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Precision medicine: a phenotypic perspective on drug safety through clinical trials in hospitalized patients

THÈSE

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Les Facultés de médecine et des sciences, sur le préavis de Monsieur J. DESMEULES, professeur ordinaire et directeur de thèse (Section des sciences pharmaceutiques et Faculté de médecine, Département d'anesthésiologie, pharmacologie et soins intensifs), Madame C. SAMER, professeure assistante et codirectrice de thèse (Faculté de médecine, Pharmacologie et Toxicologie cliniques, Hôpitaux Universitaires de Genève), Madame V. ROLLASON, docteure, privat-docent et codirectrice de thèse (Faculté de médecine, Pharmacologie et Toxicologie cliniques, Hôpitaux Universitaires de Genève), Monsieur E. ALLÉMANN, professeur ordinaire (Section des sciences pharmaceutiques), Monsieur C.B. EAP, professeur associé (Unité de pharmacogénétique et psychopharmacologie clinique, Hôpital de Cery, Prilly - Faculté de Biologie et de Médecine, Université de Lausanne, Lausanne), Madame V. NICOLAS, docteure (Department of Translational Medicine at Debiopharm International SA, Lausanne), autorisent l'impression de la présente thèse, sans exprimer d'opinion sur les propositions qui y sont énoncées.

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“Success is not final, failure is not fatal, it is the courage to continue that counts.”

Winston Churchill

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SCIENTIFIC COMMUNICATIONS

Research articles:

- **Lenoir C**, El Biali M, Luthy C, Grosgurin O, Desmeules JA, Rollason V. Snapshot of proton pump inhibitors prescriptions in a tertiary care hospital in Switzerland: less is more?. Int J Clin Pharm. 2019 Dec;41(6):1634-1641. **Impact Factor (IF) 2.054**
- **Lenoir C**, Boumaïza S, Ing Lorenzini KR, Boulvain M, Desmeules JA, Rollason V. Outcomes of drug exposition during pregnancy: Analysis from a teratology information service. Eur J Obstet Gynecol Reprod Biol. 2020 Apr;247:42-48. **IF 2.435**
- **Lenoir C**, Daali Y, Rollason V, Curtin F, Gloor Y, Bosilkovska M, Walder B, Gabay C, Nissen MJ, Desmeules JA, Hannouche D, Samer CF. Impact of Acute Inflammation on Cytochromes P450 Activity Assessed by the Geneva Cocktail. Clin Pharmacol Ther. 2021 Jun;109(6):1668-1676. **IF 6.889**
- **Lenoir C,** Terrier J, Gloor Y, Curtin F, Rollason V, Desmeules JA, Daali Y, Reny JL, Samer CF. Impact of SARS-CoV-2 infection (COVID-19) on cytochromes P450 activity assessed by the Geneva cocktail. Clin Pharmacol Ther. 2021 Nov;110(5):1358-1367. **IF 6.889**
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- **Lenoir C**, Daali Y, Doffey Lazeyras F, Gloor Y, Bosilkovska M, Curtin F, Rollason V, Walder B, Fournier R, Gabay C, Nissen M, Desmeules J, Hannouche D, Samer C. Impact of acute inflammation on cytochromes P450 activity measured with dried blood spot. *American Society of Clinical Pharmacology and Therapeutics (ASCPT) Meeting, 8-12 and 15-17 March 2021, online*. **Presidential Trainee Award.**
- **Lenoir C**, Terrier J, Gloor Y, Curtin F, Rollason V, Desmeules JA, Daali Y, Reny JL, Samer CF. Impact of COVID-19 in cytochromes P450 activity assessed by the Geneva cocktail. *European Association for Clinical Pharmacology and Therapeutics (EACPT) Meeting, 28-29 June 2021, online*.
- **Lenoir C**, Niederer A, Rollason V, Desmeules JA, Daali Y, Samer CF. Development of a PBPK model of CYP3A and CYP2C19 downregulation by interleukin-6 and esomeprazole: prediction of disease-drug and drug-drug interactions. *EACPT Meeting, 28-29 June 2021, online.*

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Poster presentations [1]:

- **Lenoir C**, El Biali M, Lutthy C, Grosgurin O, Desmeules J, Rollason V. Snapshot of the overprescription of proton pump inhibitors in a tertiary care hospital. *International Society of Pharmacovigilance (ISOP) Meeting, 11-14 November 2018, Geneva, Switzerland.*
- **Lenoir C**, Boumaïza S, Boulvain M, Ing Lorenzini KR, Desmeules J, Rollason V. Outcomes of drug exposition during pregnancy: Analysis from a teratology information service. *ISOP Meeting, 11-14 November 2018, Geneva, Switzerland.*
- **Lenoir C**, Daali Y, Rollason V, Curtin F, Gloor Y, Doffey-Lazeyras F, Bosilkovska M, Walder B, Gabay C, Nissen MJ, Desmeules JA, Hannouche D, Samer C. Impact of acute inflammation on cytochromes P450 activity measured with dried blood spot. *14ème Journée et Prix de la Recherche Clinique, 6 Mai 2021, Genève, Suisse*.

[1] Presentating authors are in bold.

ABBREVIATIONS

AAG	1-acid glycoprotein
AAT	α1-antitrypsin
ABC	ATP binding cassette
ABMR	Antibody-Mediated Rejection
ACE	Angiotensin-Converting-Enzyme
ACODs	Anticoagulants Oraux Directs
ACS	Acute Coronary Syndrome
ADME	Absorption Distribution Metabolism Elimination
ADRs	Adverse Drug Reactions
AF	Atrial Fibrillation
AhR	Aryl Hydrocarbon Receptor
AIDS	Acquired Immunodeficiency Syndrome
ALAT	Alanine Transaminase
ALL	Acute Lymphoblastic Leukemia
APP	Acute Phase Protein
ARB	Angiotensin II Receptor Blocker
AS	Alendronate Sodium
AS (genotype)	Activity Score
ASA	Acetylsalicylic Acid
ASAT	Aspartate Transaminase
ATC	Anatomical Therapeutic Chemical
ATP	Adenosine Triphosphate
AUC	Area Under the Curve
$AUC_{fexofenadine}$	$AUC_{0\text{-}6h}$ of fexofenadine
AVK	Anti-Vitamin K
BCG	tuberculosis vaccination
bid	twice daily
BMI	Body Mass Index
B/P	Blood-to-plasma partition ratio
CAR	Constitutive Androstane Receptor
CBD	Cannabidiol
CCER	Regional Research Ethics Committee
CD	Crohn's disease
CHB	Chronic Hepatitis B

CHC	Chronic Hepatitis C
CHO	Chinese Hamster Ovary
CI95%	Confidence Interval 95%
Cl_{int}	*in vitro* Intrinsic Clearance
Cl_{iv}	Intravenous Clearance
Cl_R	Renal Clearance
CLZ	Clozapine
CL/F	Oral Clearance
Cmax	Maximal Concentration
CNVs	Copy Number Variants
COLD	Chronic Obstructive Lung Disease
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Coronavirus Disease 2019
CP	Cyclophosphamide
CPIC	Clinical Pharmacogenetics Implementation Consortium
CPK	Creatine-Phosphokinase
CrCl	Creatinine Clearance
CRP	C-Reactive Protein
CS	Chondroitin Sulfate
CXP	Cell Exit Potential
CyA	Cyclosporin A
CYPs	Cytochromes P450
C/D	Concentration/Dose
C/EBP	CCAAT/Enhancer-Binding Proteins
C_{2h}	Concentration 2h after drug's administration
DBS	Dried Blood Spot
DDA	Drug-Drug-Adverse drug reaction
DDIs	Drug-Drug Interactions
DDGIs	Drug-Drug-Gene Interactions
DDGDIs	Drug-Drug-Gene-Disease Interactions
DEM	Dextromethorphan
DGIs	Drug-Gene Interactions
DGGI	Drug-Gene-Gene Interaction
DILIs	Drug-Induced Liver Injuries
DME	Drug Metabolizing Enzymes
DMET	Drug Metabolizing Enzymes and Transporters
DNA	Deoxyribonucleic Acid

DOACs	Direct Oral Anticoagulants
DOR	Dextrorphan
DP	Declustering Potential
DR4	Direct Repeat 4
DVT	Deep Vein Thrombosis
ECs	Environmental Chemicals
EDTA	Ethylenediamine Tetraacetic
EMA	European Medicine Agency
ERMBT	Erythromycin Breath-Tests
ER8	Estrogen Receptor Element
ESR	Erythrocyte Sedimentation Rate
ESRD	End-Stage Renal Disease
FDA	Food and Drug Administration
FMOs	Flavin-containing monooxygenase
fu,gut	Unbound Fraction of Drug in Enterocytes
fumic	Fraction of Unbound Drug in the *in vitro* Microsomal Incubation
fu,p	Fraction Unbound in Plasma
FXa	Factor Xa
GBT	^{13}C-Galactose Breath Test
GFR	Glomerular Filtration Rate
GGT	Gamma-Glutamyltransferase
GI	Gastrointestinal
GR	Glucocorticoid Receptor
GST	Glutathione S-Transferases
HA	Hxdrocodone-Acetominophen
HCG-β	Human Chorionic Gonadotropin-beta
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLM	Human Liver Microsome
HNF4α	Hepatocyte Nuclear Factor 4α
H. *pylori*	Helicobacter *pylori*
HWE	Hardy-Weinberg Equilibrium
IBD	Inflammatory Bowel Disease
ICH	International Council of Harmonization
ICSRs	Individual Case Safety Reports
ICU	Intensive Care Unit
IC50	Half Maximal Inhibitory Concentration

IndC50	Test Compound Concentration that supports Half Maximal Induction/Suppression
Ind_{max}	Maximal Fold Induction/Suppression Over Vehicle
IFN-γ	Interferon-Gamma
IL	Interleukin
INN	International Nonproprietary Name
INR	International Normalized Ratio
IM	Intermediate Metabolizer
IUGR	Intrauterine Growth Restriction
IVIVE	*In vitro-in vivo* Extrapolation
Ka	First-Order Absorption Rate Constant
Kapp	Concentration of Mechanism-Based Inhibitor Associated with Half Maximal Inactivation Tate
kinact	Inactivation Rate of the Enzyme
Km	Michaelis-Menten Constant
LC	Liquide Chromatography
LKM-1	Liver Kidney Microsomal type 1
LMWH	Low-Molecular-Weight Heparin
LoFA	Loss-of-Function Allele
LPS	Lipopolysaccharide
MDR/TAP	Multidrug Resistance/Transporters of Antigen Presentation
MDZ	Midazolam
MEGX	Monoethylglycinexylide
MGB	Minor Groove Binder
MIPD	Model-informed Precision Dosing
miRNA	micro Ribonucleic Acid
MODS	Multiple Organ Dysfunction Score
MOF	Multiple Organ Failure
MR	Metabolic Ratio
MRM	Multiple Reaction Monitoring
mRNA	messenger Ribonucleic Acid
MS/MS	Tandem Mass Spectrometry
m/z	Mass-to-charge ratio
NA	Not Applicable
NAFLD	Non-alcoholic Fatty Liver Disease
NASH	Non-alcoholic Steatohepatitis
NCE	New Chemical Entity

NCLZ	Norclozapine
NCR	Non Clinically Relevant
NFQ	Non-fluorescent Quencher
NF-κB	Nuclear Factor-kappa B
NICE	National Institute for Health and Care Excellence
NID	Non-Insulin Dependent
NM	Normal Metabolizer
NO	Nitric-Oxide
non-DHP CCBs	Non-Dihydropyridine Calcium Channel Blockers
NQOs	NAD(P)H:quinone oxidoreductases
NS	Non-Significant
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
NVAF	Nonvalvular Atrial Fibrillation
od	once daily
OMPZ	Omeprazole
OMS	Organisation Mondiale de la Santé
OTC	Over-The-Counter
p	P-value
PAIs	Platelet Aggregation Inhibitors
PASI	Psoriasis Area Severity Index
PBPK	Physiologically Based Pharmacokinetics
PBREM	Phenobarbital-Responsive Enhancer Module
PCR	Polymerase Chain Reaction
PD	Pharmacodynamic
PDE5	Phosphodiesterase 5
PDE5is	Phosphodiesterase 5 inhibitors
PE	Pulmonary Embolism
Peff,man	Human jejunum effective permeability
PICOS	Participants, Interventions, Comparisons, Outcomes, Study design
PK	Pharmacokinetic or pharmacocinétique
PM	Poor Metabolizer
PPAR	Peroxisome Proliferator-Activated Receptor
PPI	Proton Pump Inhibitor
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PT (MedDRA)	Preferred Term
PT	Prothrombin Time
PXR	Pregnane X Receptor

P-gp	P-glycoprotein
qPCR	Quantitative Polymerase Chain Reaction
RA	Rheumatoid Arthritis
RAHS	Rebound Acid Hypersecretion
RM	Rapid Metabolizer
RNAse	Ribonuclease
rs ID	Reference SNP Identifier
RT-PCR	Real Time-Polymerase Chain Reaction
RXR	Retinoid X Receptor
SARS-CoV-2	Serious Acute Respiratory Syndrome Coronavirus 2
SD	Standard Deviation
SE	Standard Error
SLC	Solute Carrier
SLE	Systemic Lupus Erythematosus
SmPCs	Summary of Product Characteristics
SNPs	Single Nucleotide Polymorphisms
SOC	System Organ Class
SRAS-CoV-2	Coronavirus 2 du Syndrome Respiratoire Aiguë Sévère
SSPTC	Swiss Society for Clinical Pharmacology and Toxicology
SSRIs	Selective Serotonin Reuptake Inhibitors
SULT	Sulfo-Transferases
TA	Tranexamic Acid
TC	Tramadol Chlorhydrate
TDM	Therapeutic Drug Monitoring
TIS	Teratology Information Service
TLR	Toll-Like Receptor
TNF-α	Tumor Necrosis Factor alpha
T1D	Type I Diabetes
$t_{1/2}$	half-life
T2D	Type II Diabetes
UGT	Uridine 5'-Diphospho-Glucuronosyltransferases
UK	United Kingdom
UM	Ultra-rapid Metabolizer
UMC	Uppsala Monitoring Center
US	United States
Vd	Volume of Distribution
Vmax	Maximum rate of metabolism

Vss	Volume of Distribution at Steady State
VTE	Venous Thromboembolism
WHO	World Health Organization
XREM	Xenobiotics-Responsive Enhancer Module
1-OH-MDZ	1-hydroxymidazolam
4βOHC	4β-hydroxycholesterol
5-OH-OMPZ	5-hydroxyomeprazole
7-HC	7-Hydroxycoumarine
Ω	Omega
χ^2	Chi-squared

RÉSUMÉ

Au cours des dernières décennies, l'approche thérapeutique « *one size fits all* » communément appliquée a été progressivement abandonnée au profit d'une médecine centrée sur le patient, bien qu'Hippocrate y fît déjà référence il y a plus de deux mille ans. La médecine de précision vise à garantir une meilleure efficacité et sécurité des traitements en prenant en compte les variabilités intra- et interindividuelles. Elles sont la résultante de l'effet combiné du génome et de l'exposome qui vont influencer le profil pharmacocinétique (PK) et/ou pharmacodynamique d'un médicament. La variabilité PK se manifeste principalement au niveau du métabolisme, et notamment des cytochromes P450 (CYPs), enzymes responsables de la métabolisation de près de trois quarts des médicaments commercialisés. La P-glycoprotéine (P-gp) est par ailleurs un transporteur clé impliqué dans les étapes d'absorption, de distribution et d'élimination des médicaments. Plusieurs facteurs génétiques, environnementaux, physiologiques et physiopathologiques influencent l'expression et l'activité des CYPs et de la P-gp, ce qui peut être aisément responsable de la variabilité de l'efficacité et de la toxicité des médicaments.

L'objectif de cette thèse est d'étudier l'impact des polymorphismes génétiques et des facteurs environnementaux (interactions médicamenteuses), physiologiques (âge, genre, indice de masse corporel…) et physiopathologiques (inflammation) sur l'activité des CYPs et de la P-gp et leur impact sur la PK de certains médicaments qui en sont substrats. Cette thèse est divisée en trois volets majeurs : l'effet du génotype et du phénotype du CYP3A/P-gp sur la PK des anticoagulants oraux directs (ACODs), l'effet des maladies sur l'activité des CYPs (inflammation), et la prédiction *in silico* des interactions maladie-médicament (inflammation) et médicament-médicament (ésoméprazole) sur les CYPs. Cette recherche souligne l'influence des différentes sources de variabilité et la nécessité de les intégrer pour améliorer l'efficacité et la sécurité des traitements.

Le **premier chapitre** est une introduction générale qui présente le concept de médecine de précision dans le contexte des variabilités intra- et interindividuelles de l'efficacité et de la sécurité des médicaments. Les causes et les conséquences de ces variabilités sont explorées, et plus précisément l'impact des facteurs génétiques, environnementaux (interactions médicamenteuses, alimentation, consommation de produits toxiques), physiologiques (âge, grossesse, genre, indice de masse corporelle) et physiopathologiques (insuffisance rénale et hépatique et inflammation) sur l'expression et l'activité des CYPs et de la P-gp. Une méthode de prédiction, la physiologie basée sur la PK (PBPK), est exposée comme un outil émergeant

de plus en plus utilisé pour la médecine de précision. De plus, les méthodes analytiques utilisées pour l'analyse des échantillons sont présentées.

Les chapitres 2 à 3 correspondent au premier volet de ce travail de thèse, à savoir l'impact du polymorphisme génétique et du phénotype du CYP3A/P-gp sur leurs substrats apixaban et rivaroxaban.

Le **deuxième chapitre** présente deux revues systématiques (**article de revue 1** et **2**) d'études et de rapports de cas d'interactions médicamenteuses avec l'apixaban et le rivaroxaban, respectivement. La base de données mondiale des effets indésirables de l'Organisation Mondiale de la Santé (OMS) a été utilisée pour analyser les évènements indésirables rapportés après des interactions médicamenteuses avec l'apixaban et le rivaroxaban. Nous avons mis en évidence que l'apixaban et le rivaroxaban ont potentiellement des interactions médicamenteuses significatives, notamment avec les modulateurs de l'activité du CYP3A et de la P-gp ou de l'hémostase.

Le **troisième chapitre** présente une étude observationnelle prospective qui a évalué l'impact du génotype et du phénotype de CYP3A et de P-gp sur l'exposition à l'apixaban et au rivaroxaban (**article de recherche 1**). Cette étude visait à déterminer si les polymorphismes génétiques et les activités phénotypiques du CYP3A/P-gp pouvaient avoir un impact significatif sur les concentrations sanguine de l'apixaban et du rivaroxaban. C'était le cas pour l'activité phénotypique de la P-gp, qui pourrait avoir un impact cliniquement pertinent sur la réponse au médicament. L'activité phénotypique de la P-gp pourrait donc être considérée comme un facteur pertinent pour l'ajustement de la dose des ACODs à l'avenir, en plus des facteurs existants.

Les chapitres 4 à 7 constituent le deuxième volet de cette thèse, à savoir l'impact de la physiopathologie (inflammation) sur l'activité des CYPs au travers de deux études cliniques et de deux revues systématiques de la littérature.

L'étude clinique présentée dans le **quatrième chapitre** (**article de recherche 2**) a été effectuée pour investiguer l'impact d'une inflammation aigue sur l'activité des CYPs au fil du temps. Cette étude prospective observationnelle à évaluer la variation des activités des six principaux CYPs avant et les jours suivants une chirurgie élective de la hanche chez trente patients. L'étude a mis en évidence que l'activité des CYP3A, 2C19 et 1A2 ont diminué, que l'activité des CYP2B6 et 2C9 ont augmenté et que l'activité du CYP2D6 n'a pas varié après la

chirurgie. Ces variations se sont produites à des magnitudes et cinétiques différentes, avec un effet maximal à des jours différents selon le CYP.

Dans le **cinquième chapitre**, une seconde étude prospective observationnelle a été conduite chez trente patients hospitalisés en raison de la maladie à coronavirus 2019 (COVID-19) (**article de recherche 3**) car ils présentaient un syndrome inflammatoire aigue. Les activités des CYPs ont été mesurées pendant et trois mois après l'infection par le coronavirus 2 du syndrome respiratoire aiguë sévère (SRAS-CoV-2) afin de comparer leurs phénotypes de bases avec ceux observés au cours de la maladie. Les mêmes variations d'activité des CYPs qu'avec la chirurgie ont été retrouvées, mais à des magnitudes différentes.

Le **sixième chapitre** présente une revue systématique (**article de revue 3**) d'articles et de rapports de cas sur l'impact de l'inflammation sur l'activité des CYPs chez les adultes. Les résultats de 218 publications ont été résumés et divisés en quatorze sources différentes d'inflammation. Cette revue avait pour objectif d'illustrer l'importance des interactions maladie-médicament dans l'individualisation des traitements, et leurs différentes conséquences selon la maladie inflammatoire sous-jacente et l'isoenzyme impliqué.

Le **septième chapitre** est consacré à l'impact de l'inflammation dans la population pédiatrique. Cette revue systématique (**article de revue 4**) visait à mettre en évidence la complexité supplémentaire de l'impact de l'inflammation sur les CYPs lorsqu'elle est associée à la notion de développement. En effet, des impacts différents des maladies sur l'activité des CYPs en fonction de l'âge et de l'isoenzyme considéré ont été mis en évidence à travers vingt-sept articles. Les études cliniques conduites en pédiatrie sont rares et les efforts futurs devraient tendre vers une meilleure individualisation des traitements dans cette population spéciale.

Le **huitième chapitre** (et dernier volet de cette thèse) porte sur la prédiction des interactions combinées maladie-médicament sur les médicaments substrats des CYPs. Nous avons mené une étude *in silico* (prédiction PBPK) pour modéliser l'impact de l'interleukin-6 (IL-6) (interaction maladie-médicament) et de l'ésoméprazole (interaction médicamenteuse) sur l'activité du CYP3A et du CYP2C19 (**article de recherche 4**). Les modèles pour le midazolam, le 1-OH-midazolam, l'oméprazole, le 5-OH-oméprazole, l'ésoméprazole et l'IL-6 ont été pris directement ou adaptés de la littérature puis validés. La vérification de leur applicabilité a été effectuée grâce aux données présentées dans le chapitre 4.

Le **neuvième chapitre** présente une discussion générale des résultats de cette thèse et les conclusions et perspectives qui peuvent en être tirées.

Enfin, le **dixième chapitre** comprend des publications qui sortent du cadre de cette thèse et sont donc présentées en annexe. Il s'agit de deux études observationnelles portant sur l'adéquation de la prescription des inhibiteurs de la pompe à protons dans un hôpital tertiaire (**article de recherche 5**) et les conséquences fœtales et néonatales de l'exposition médicamenteuse pendant la grossesse (**article de recherche 6**).

ABSTRACT

In recent decades, there has been a gradual shift from the commonly applied « one size fits all » therapeutic approach to patient-centered medicine, although Hippocrates already referred to it over two thousand years ago. Precision medicine aims to ensure better treatment efficacy and safety by taking into consideration intra- and inter-individual variabilities. They are the result of the combined effect of the genome and the exposome that will influence the pharmacokinetic (PK) and/or pharmacodynamic profile of a drug. PK variability is mainly at the level of metabolism, and in particular of cytochromes P450 (CYPs), the enzymes responsible for the metabolism of nearly three quarters of marketed drugs. P-glycoprotein (P-gp) is furthermore a key transporter involved in drugs absorption, distribution, and elimination steps. Several genetic, environmental, physiological, and pathophysiological factors influence the expression and activity of CYPs and P-gp, which may be largely responsible for the variability of drug efficacy and toxicity.

The objective of this thesis is to study the impact of genetic polymorphisms and environmental (drug-drug interactions), physiological (age, gender, body mass index...) and pathophysiological (inflammation) factors on the activity of CYPs and P-gp and their impact on the PK of some of their drug substrates. This thesis is divided into three major parts: the effect of CYP3A/P-gp genotype and phenotype on the PK of direct oral anticoagulants (DOACs), the effect of diseases (inflammation) on CYPs activity, and *in silico* prediction of disease-drug (inflammation) and drug-drug (esomeprazole) interactions on CYPs. This research highlights the influence of different sources of variability and the need to integrate them to improve the efficacy and safety of treatments.

The **first chapter** is a general introduction to present the concept of precision medicine in the context of intra- and inter-individual variabilities in drug efficacy and safety. The causes and consequences of these variabilities are explored, and more specifically the impact of genetic, environmental (drug-drug interactions, diet, toxic products), physiological (age, pregnancy, gender, body mass index) and pathophysiological (renal and hepatic insufficiency and inflammation) factors on the expression and activity of CYPs and P-gp. A predictive method, the physiological based-PK (PBPK), is an emerging tool increasingly used for precision medicine. In addition, analytical methods used for sample analysis are presented.

Chapters 2 to 3 correspond to the first part of this thesis work, namely the impact of genetic polymorphism and phenotype of CYP3A/P-gp on their substrates apixaban and rivaroxaban.

The **second chapter** presents two systematic reviews (**review article 1** and **review article 2**) of articles and case reports of drug-drug interactions (DDIs) with apixaban and rivaroxaban, respectively. The World Health Organization (WHO) Global Adverse Reaction Database was used to analyze adverse events reported after DDIs with apixaban and rivaroxaban. We pointed out that apixaban and rivaroxaban have potentially significant DDIs, including with modulators of CYP3A and P-gp activity or hemostasis.

The **third chapter** presents a prospective observational study that assessed the impact of CYP3A and P-gp genotype and phenotype on apixaban and rivaroxaban exposure (**research article 1**). The purpose of this study was to determine whether CYP3A/P-gp genetic polymorphisms and phenotypic activities could have a significant impact on apixaban and rivaroxaban blood concentrations. This was the case for P-gp phenotypic activity, which could have a clinically relevant impact on drug response. P-gp phenotypic activity could therefore be considered a relevant factor for DOACs dose adjustment in the future, in addition to existing factors.

Chapters 4 to 7 constitute the second part of this thesis, namely the impact of pathophysiology (inflammation) on CYPs activity through two clinical studies and two systematic reviews of the literature.

The clinical study presented in the **fourth chapter** (**research article 2**) was conducted to investigate the impact of acute inflammation on CYPs activity over time. This prospective observational study evaluated the variation of the activities of the six main CYPs before and the days following elective hip surgery in thirty patients. The study showed that CYP3A, 2C19 and 1A2 activity decreased, CYP2B6 and 2C9 activity increased and CYP2D6 activity did not vary after surgery. These changes occurred at different magnitudes and kinetics, with a maximum effect on different days depending on the CYP.

In the **fifth chapter**, a second prospective observational study was conducted in thirty hospitalized coronavirus disease 2019 (COVID-19) patients (**research article 3**) as they present an acute inflammatory syndrome. CYPs activities were measured during and three months after serious acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection to compare their baseline phenotypes with the ones observed during the course of the disease. The same modulation of CYP activities as with surgery was found, but with different magnitudes.

The **sixth chapter** presents a systematic review (**review article 3**) of articles and case reports on the impact of inflammation on CYPs activities in adults. The results of 218 publications were summarized and divided into fourteen different sources of inflammation. The aim of this review was to illustrate the importance of disease-drug interactions in the individualization of treatments, and their different consequences depending on the underlying inflammatory disease and the isoenzyme involved.

The **seventh chapter** is focusing on the impact of inflammation in the pediatric population. This second systematic review (**review article 4**) aimed to highlight the additional complexity of the impact of inflammation on CYPs when combined with the notion of development. Indeed, different impacts of diseases on CYPs activity according to age and to the considered isoenzyme were highlighted through twenty-seven articles. Clinical studies conducted in pediatrics are rare and future effort should aim to better individualize treatments in this special population.

The **eighth chapter** (and last part of this thesis) focuses on the prediction of combined disease-drug interactions on CYPs substrate drugs. We conducted an *in silico* study (PBPK prediction) to model the impact of interleukin-6 (IL-6) (disease-drug interaction) and esomeprazole (DDIs) on CYP3A and 2C19 activities (**research article 4**). Models for midazolam, 1-OH-midazolam, omeprazole, 5-OH-omeprazole, esomeprazole, and IL-6 were taken directly or adapted from the literature and then validated. Verification of their applicability was performed using the data presented in chapter 4.

The **ninth chapter** presents a general discussion of the results of this thesis and the conclusions and perspectives that can be drawn from them.

Finally, the **tenth chapter** includes publications that are outside the scope of this thesis and are therefore presented in the appendix. These are two observational studies on the appropriateness of prescribing proton pump inhibitors in a tertiary hospital (**research article 5**) and the fetal and neonatal consequences of drug exposure during pregnancy (**research article 6**.

Chapter 1: General Introduction.

1.1 Precision medicine

Precision or personalized medicine principles are a thousand years old, having begun to emerge with Hippocrates, the Greek physician known as the « Father of Western Medicine », who said « *give different ones [therapeutic drinks] to different patients, for the sweet ones do not benefit everyone, nor do the astringent ones, nor are all the patients able to drink the same things* » and « *it is much more important to know what sort of patient has a disease than what sort of disease a patient has* » [1]. Later, Archibald Garrod, an English physician, was the first to discover that individuals are widely different with respect to their metabolism and that this could explain inter-individual variability in terms of susceptibility to disease and its manifestations [2]. More recently, the provision of a significant budget for precision medicine programs by the United States (US) (2015) and China (2016), represents a direct continuation for the development of precision medicine [3].

A gradual shift toward patient-centered healthcare has been observed over the last several decades [4]. Indeed, the propensity to personalized medicine has been facilitated by the understanding of human health and disease, which was allowed by initiatives such as the Human Genome Project and Human Proteome Project launched in 1988 and 2010, respectively [5]. They enabled detailed analysis of the relatively static human genome and the extremely dynamic human proteome, respectively [5]. The proteome contains over a 1000-fold more cellular information than the genome because it has the adaptive capacity to capture the dynamic changes in biology and function within an individual, either due to disease, drug treatment or baseline physiological within-person variation [5,6]. The Human Proteome Project was over 90% completion in 2020 but the Human Genome Project was completed in 2003 with the publication of the first sequence of the human genome [5,7]. It revealed 20’000-25’000 genes and was followed by the HapMap project and the 1000K Genome Project, to describe the broad range of human genetic variations [3,8]. Indeed, environmental and evolutionary factors lead to unequal biological make-up between humans [9].
The wide development in recent years of molecular biology and genomics made genetic tests more available and its pairing with the emergence of areas such as metabolomics and proteomics led to the increase in targeted therapies and identification of biomarkers [3]. As a result, an unprecedented aptitude for prevention, detection and treatment of disease was generated, transforming medicine [3,10].

The global purpose of precision medicine is to select a drug with the best benefit/risk balance [11]. It has been estimated that 40-70% of patients have already experienced an efficacy or a safety concern with their pharmacological therapy [12]. Therefore, variation in drug response

can range from inadequate therapeutic efficacy to serious adverse drug reactions (ADRs), leading to unbeneficial response to a treatment [13,14]. For instance, it has been estimated that only 25-60% of patients take advantage of their prescribed treatment in a large number of therapeutic areas [14]. Another example is that the top ten highest-grossing drugs in the US in 2015 failed to improve the condition of between 3 to 24 patients for one single patient they do help [15].

On the other hand, ADRs are estimated to be the fourth leading cause of death [16,17]. The risk of ADRs occurrence grows with the misuse of drugs, notably characterized by inappropriate prescriptions or drug-drug interactions (DDIs) [17,18]. ADRs can be caused by many factors and it is essential to identify all of them, as poor management and monitoring of ADRs results in unnecessary hospitalization, morbidity and mortality [18].

There are two periods in the life-cycle of a drug where ADRs can be detected. Clinical trials ensure that a drug is safe and effective for its intended use when approved on the market but post-marketing adverse event collection guarantees that drug's safety information is always up to date [19]. Indeed, new ADRs can appear after approval due to small clinical trial samples and strict selection of patients [18]. The principle allowing the extensive screening of ADRs and efficacy concerns is called pharmacovigilance [18]. Pharmacovigilance is defined by World Health Organization (WHO) as the « science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problems » [20]. This concept has evolved a lot since its origin in 1938 and the creation of the US Federal Food, Drug and Cosmetic Act due to a thousand of deaths following the use of diethyl glycol as a solvent [20,21]. The thalidomide tragedy is the other triggering event, since it led to the creation of the pharmacovigilance systems in Europe and the WHO program for international drug monitoring in 1968 [21].

The capacity to collect pharmacovigilance data has extensively increased due to the rise of computer technology [21]. For instance, an exponential increase in individual case safety reports (ICSRs) is observed in the WHO global database of ICSRs, named VigiBase, since its creation in 1967, partly explained by the legal requirements for ADRs reporting implemented in several countries [21]. Also, pharmaceutical industries have a legal obligation to create an ICSR from each adverse event associated with any of their marketed products [22]. The emergence of large databases and computerized automated statistical analyses has deeply modified the assessment of drug safety and benefit/risk balance [21]. Spontaneous reporting is the main resource to identify the existence of new ADRs not predicted by pharmacology [23]. Although a single ICSR can rarely provide sufficient information to make regulatory decisions, as it only reveals a probable association between a drug with a certain reaction, multiple similar ICSRs can generate a signal [23]. A signal is defined as « a new potential association or new aspects of a known association between drugs and ADRs that warrant

further investigation » [21,23]. Disproportionality analyses are used to identify a potential signal [23]. It is a measure that compares the proportion of an event stated with a drug among all events reported for the drug with the same proportion for a comparator population [23]. Initially, databases were created from information on adverse events reported through standardized processes, and then signal detection activities were implemented thanks to the ability to query the databases [20]. The additional information on drug safety and efficacy brought by real-world data is discussed in further details in chapters 2 and 3.

This allows the detection of factors that mitigate the efficacy and safety of the drug when it is provided to the patient, but the ongoing trend is rather to use therapies targeting the specific identified profile of the patients [2]. By means of precision medicine, individuals at higher risk of ADRs could be identified in advance and an adapted treatment or dose could be prescribed to avoid any harm [17]. Indeed, 50-67% of ADRs could be preventable [17]. Moreover, precision medicine could help to improve treatment efficacy, as it is well-known that ADRs can lead to poor medication adherence or discontinuation of therapy, depending on their impact on quality of life [17].

Consequently, precision medicine is a leading principle in evolution of medicine, aiming to ensure the best efficacy and safety by customization and adjustment of treatments [3]. It is a multicomponent strategy that manages the health according to the individual circumstance of the patient and the information from others [12,14]. Indeed, many diseases have an underlying heterogeneity, due to own biochemical, physiological and environmental exposure or behavioral profile of the individual suffering from the disease [14]. Therefore, it is now widely accepted that processes used to treat, monitor and prevent diseases have to be individualized [2].

The existence of genetic variants as an explanation for inter-individual difference in drug efficacy and safety was first suggested in 1957 by Motulsky [24]. In 1998, it was estimated that at least one genetic make-up of an individual accounts for up to 95% of responses to medication [14]. This contributes to the growing interest in pharmacogenomics [14]. The Clinical Pharmacogenetics Implementation Consortium (CPIC) aims to establish concrete pharmacogenomic associations with clinical settings and to provide evidence-based guidelines on how available genetic test results may be used to optimize treatment [14,25]. At the time of development and prescription, selection of adequate drugs and dosing could be rationalized and guided by the genetic characteristics of the patient and/or of population subgroups [3,10]. Several summary of product characteristics (SmPCs) of drugs include information about ADRs or different dose recommendations based on the genomic profile of the patient, as genetic

variants have been shown to have an impact on their metabolism [10]. Nowadays, though not exclusively related to drug metabolism, approximatively 18% of prescribed drugs in the US have a gene variant-drug response association in their SmPC [24]. However, the application of « companion » diagnostic tests is frequently required to identify patient who will benefit from such particular medicine [10]. Some patients on other drugs, such as tamoxifen, allopurinol, selective-serotonin reuptake inhibitors (SSRIs), codeine, tramadol, azathioprine, mercatopurine and some chemotherapeutic agents may benefit from prior testing before prescription, but this is dependent on the prescriber [26]. In the US, insurance companies may cover pharmacogenomic testing, but it differs according to the testing required, the indication and the insurance providers [26]. Drug metabolism concerns, medication non-adherence, self-adjustment of dosing or frequency to obtain a clinical benefit, prescription of medication with known ADR, family history of intolerance or the patient's wish for preemptive testing are the most common indications [26]. In Switzerland, some gene-drug pairs are reimbursed, depending on a clear indication for administration of the drug, the occurrence of safety or efficacy concerns scientifically related to a genetic mutation, the prescription from the « List of Swiss Society for Clinical Pharmacology and Toxicology (SSPTC) » or by a certified clinical pharmacologist [27].

Pharmacogenomics is a component of precision medicine, but a more comprehensive approach is now considered with the addition of biomarkers, lifestyle, diet and clinical data [13]. Indeed, the proteome is influenced by dynamic forces that are endogenous or exogenous to the human host [6]. Inter- and intra-individual variability in response to treatment is the result of the combined effects of genetic, physiological, pathophysiological and environmental factors that are different between individuals and/or in the same individual over a given period of time [28]. The European Union's Horizon 2020 Advisory Group defines precision medicine as the « characterization of individuals' phenotypes and genotypes for tailoring the right therapeutic strategy for the right person at the right time and/or to deliver timely and targeted prevention » [4]. Indeed, using a personalized treatment means that the « five rights of medications » (i.e. the right drug at the right dose with the right route of administration at the right time and for the right patient) have to be fulfilled [29]. The key of personalized therapies is to take into account the individual level variation of both the person and/or their disease [10].

1.2 Variability to treatment responses

The response to traditional pharmacotherapies is expressed through two general levels, i.e. the pharmacokinetic (PK) and the pharmacodynamic (PD) properties of the drug [3]. They

describe how the body interacts with the drug and how the drug interacts with the body, respectively [3]. They are two distinct entities but it is the combination of both which determines the dose-response relationship and the clinical effects of drug therapy [30,31]. Indeed, the concentration of the drug at the binding/target site is the key parameter for a drug effect [32]. The drug response is a function of dose and time and is typically nonlinear, leading to a significant level of complexity [32]. Moreover, PK and PD are under genetic, environmental, pathophysiological, and physiological controls, leading to DDIs that may be both PK and PD [33,34]. Indeed, a DDI is defined as « the pharmacological or clinical response to the administration or co-exposure of a drug with another substance that modifies the patient's response to the drug » [33]. Clinically significant DDIs are a therapeutic challenge encountered by many drugs, as they lead to serious ADRs or will reduce the efficacy of some drugs [33,35]. For instance, it has been reported that DDIs leads to 20-30% of all reported ADRs, with an increase in percentage among older patients who take more than two concomitant treatments [33,35]. A DDI is considered clinically significant when [33]:

- Drug elimination occurs primarily through a single metabolic pathway
- A drug is a potent modulator of a drug-metabolizing enzyme (DME)
- One or both interacting drugs has/have a steep dose-response curve or a narrow therapeutic index
- Inhibition of the primary metabolic enzyme or induction of a secondary metabolic enzyme results in diversion of the drug into an alternative pathway, which generates a metabolite that has toxic or modified PD activity
- Drug has an original or acquired nonlinear PK
- The drug is metabolized through or inhibits a polymorphic DME

These criteria could be classified as PK or PD interactions or both [35]. That is why correct administration of drugs in a special population should be established on both variations in PK and PD behaviors as compared to normal individuals [36]. A good knowledge of causes and consequences contributes to avoid DDIs [35].

PD interactions occur when two drugs or substances have similar molecular targets, leading to additive/synergic (agonist) or opposing (antagonist) effects, without affecting the PK parameters of each other [33]. It is more difficult to accurately quantify them because clinical impact is more elusive than with PK [30]. Intrinsic and extrinsic factors affect PD, including the density of receptors on the cell surface, the process of signal transmission by secondary messengers, and factors that control gene translation and protein production [30,32]. Beyond the agonist and antagonist effects, a ligand could have other functions such as partial agonism (both agonist and antagonist), inverse agonism, reversible competitive antagonism, irreversible competitive antagonism or non-competitive antagonism (allosteric modulators)

[32]. A drug action is also defined by the specificity, selectivity and affinity of a receptor to a ligand, but also the potency and the efficacy of the drug [32]. Potency describes the relationship between drug dose and the magnitude of the effect, while efficacy is the maximal response [32]. Finally, duration of effect is determinant and is related to the time that a drug is engaged on the receptor and on intracellular signaling and gene regulation [30]

A PK DDI involves one drug or substance altering at least the absorption, distribution, metabolism, or elimination (ADME) of another drug or substance [33].

Absorption is the transport of the drug from the site of administration to the systemic circulation by passive diffusion, convective transport, active transport, facilitated transport, ion pair transport and pinocytosis [30,37]. Several factors can impact absorption and, thus interact with the drug and change its bioavailability and PK [33,37]. Moreover, the gastrointestinal (GI) tract contains drug-metabolizing enzymes and transporters (DMET) that should be considered because GI metabolism can alter the absorption of orally administered drug that do not have to be bioactivated (prodrug) [33]. For example, P-glycoprotein (P-gp), a multidrug efflux transporter, can lead to chemotherapy resistance as it is expressed in many tissue barriers e.g. in the intestine, the liver, the kidney [35]. Moreover, cytochromes P450 (CYPs) are markedly expressed in enterocytes present in the epithelium of the small intestine and can reduce the oral bioavailability of drugs [38]. This phenomenon is called « first-pass metabolism » and could also occurs in the liver through the passage of the drug into the hepatic portal system before reaching the systemic circulation [38]. Absorption is a limiting step for drug efficacy as 40% of commonly used drugs have a bioavailability under 50% [38].

Drug distribution to its target site in sufficient amount is the next prerequisite for a drug to exert its therapeutic effect [33,37]. The unbound fraction of a drug is pharmacologically active and binding to plasma proteins (albumin, glycoproteins and intracellular proteins) is thus one of the key parameters of distribution [33,37]. The binding to plasma protein is linked to the volume of distribution (Vd) of a drug, and thus the distribution through body tissues [39]. The drug displacement from blood components or tissue-binding sites increases the apparent Vd and therefore drug efficacy [33].

The liver is the first site of metabolism, even though metabolism can start at the absorption site, as previously mentioned [33,37]. DME modify the drug, and can be induced or inhibited by several factors or could be a competitive site for two drugs [33,37]. Phase I enzymes are responsible for a step of metabolism which consists of oxidation, reduction or hydrolysis of primarily lipophilic xenobiotics to produce more polar water-soluble molecules [33]. Oxidation,

reduction and hydrolysis are known as pre-conjugation and the process generally adds an oxygen molecule, a hydrogen molecule and water, respectively [37]. Phase I enzymes involve flavin-containing monooxygenase (FMOs), NAD(P)H:quinone oxidoreductases (NQOs), amine oxidases, alcohol dehydrogenases, esterases, peroxidases, and the CYP superfamily as major contributors [33]. The polar groups added to the xenobiotics make it possible to be directly eliminated or conjugated by phase II enzymes, leading to more hydrophilic and heavier compounds that are not able to diffuse across phospholipid membrane barrier [33]. The anionic groups added act as affinity tags for elimination transporters [33]. Phase II enzymes are sulfo-, methyl-, glutathione-, acetyl- or UGT (UDP-glucuronosyltransferase) [33]. Differences in drug metabolism among patients are frequently considered as the major contributors to inter-individual variability in drug response [38].

Final step of the ADME process is elimination, which principally occurs via liquid elimination through renal and biliary excretion [37]. Renal excretion of unchanged drugs is the major route of elimination for 25-30% of drugs [39]. The directional transport of drug across organs requires drug uptake transporters as well as efflux transporters [33]. Elimination involves four important quantitative concepts, i.e. clearance (rate of elimination), plasma half-life or total-body half-life (time required to reduce by 50% the amount in the body), first-order kinetics and zero-kinetics order (constant fraction of drug eliminated per unit of time) [37]. A change in transporters activity, as well as a modification of the glomerular filtration rate (GFR) could have an impact on drug elimination [33].

PK mechanisms mediate the most clinically significant DDIs [40]. Indeed, the inter-individual variability in PK can lead to a variation in plasma drug concentration over time by about 600-fold when the same drug dosage is administered to individuals with the same weight [41]. The emergence of the basic principles of PK and their identification as the cornerstone for achieving the optimal drug profile dates from 1538, when Paracelse said that « *only the dose makes the poison* » [32]. Indeed, to have a good benefit/risk ratio, drug concentrations must be within the therapeutic window [37,38]. But the ADME process undergoes major inter- and intra-individual variations during lifetime due to the effect of genetic, epigenetic, environmental and physiological or pathophysiological factors [42].
Genes families involved in ADME are highly polymorphic and it has been stated that 15-30% of inter-individual differences observed in drug metabolism and response to a drug are linked to genetic factors [24,41,43]. A genetic polymorphism is defined as a mutation that occurs in more than 1% of a population and the consideration of the population studied is important for drug response predictions because many polymorphism are population-specific [24,44]. The identification of the variations of DMET can help optimizing safety and efficacy, as it may allow

the detection of patients who are at greater risk of ADR or who would benefit from dose adjustment [4]. That is why each research project of this thesis is focused on the causes and/or consequences of the variability in DMET activities.

1.3 Cytochromes P450

CYPs are the major phase I enzyme (90-95%) and the main DME as they are responsible for approximatively three quarters of drug metabolism, as illustrated in Figure 1 [42,45].

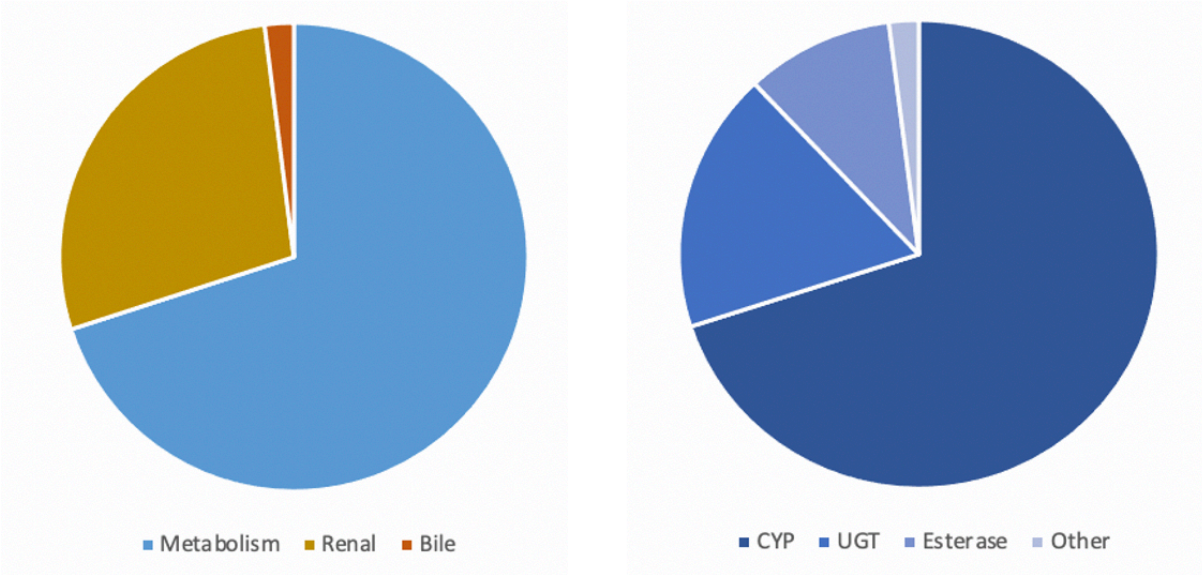

Figure 1: Percentage of drugs eliminated via metabolism and relative importance of CYP in this process. Adapted from [45].

CYPs are heme-thiolate monooxygenases present in all kingdoms of life [42]. They catalyze the oxidation and reduction of endogenous and exogenous chemicals, with low substrate specificity and turn-over rates [42]. In humans, they are predominantly present in the liver followed by the intestine, although present in all tissues [42]. They are also expressed in the mitochondrial inner membranes of steroidogenic tissues, such as adrenal cortex, reproductive organs, breast and placenta [46]. They do not only render the compounds more hydrophilic to facilitate their excretion, but may also have an impact on treatment outcomes by contributing to the production of active or inactive metabolites, affecting drug bioavailability and participating to drug resistance [47]. They are in the heart of the human defense system against a broad variety of environmental compounds potentially damaging for cellular metabolism and health [46]. CYPs can also lead to lethal synthesis, by the biotransformation of environment chemicals, such as drugs, additive and pollutants into reactive carcinogenic products [46].

They are also implicated in the synthesis and degradation of endogenous compounds, such as steroid hormones, and vitamins metabolism, unsaturated fatty acids oxidation and cholesterol biosynthesis [46]. Therefore, CYPs have a pivotal role in cellular metabolism and homeostasis maintenance [46]. CYPs involved in the biosynthesis of endogenous molecules are considered as moonlightening proteins, while CYPs involved in the metabolism of exogenous substances are not [46]. A moonlightening protein is defined as a protein that achieves multiple and autonomous functions that are often unrelated, and identification of moonlightening CYPs has added a new aspect in the complexity and diversity of functions catalyzed by CYPs [46].

CYPs were first studied in the 1940s *in vitro* and have been the subject of much further research in the clinical pharmacology and toxicology field [42]. However, it is necessary to be careful when data are extrapolated to human, as CYPs expression, regulation and function is highly species-specific [42]. Indeed, the thalidomide tragedy occurred because toxicological studies were conducted in mice and did not conclude for potential teratogenicity while its administration in pregnant women for morning sickness resulted in severe birth defects (phocomelia) [42]. These limb malformations were attributed to the formation of a reactive phase I metabolite in higher proportions in humans [42].

The existence of different CYP isoenzymes was first described in the late 60s and the Human Genome Project identified 57 human CYP genes and 58 pseudogenes [42]. The human CYP superfamily is divided into 18 families (first Arabic numeral) and 44 sub-families (letter) [42]. The nomenclature used to name the CYPs is described in Figure 2.

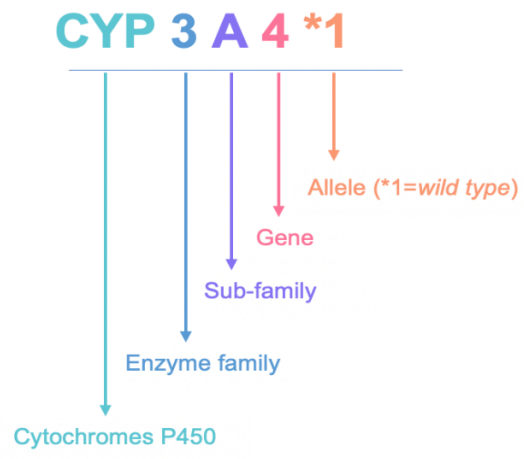

Figure 2: CYPs nomenclature.

CYP families 1-3 represent 70% of the phase I enzyme metabolism and are responsible for the vast majority of the clearance mechanisms [42,45,46]. Among all the isoenzymes identified

(second Arabic numeral), only seven contribute to CYP-specific drug metabolism with different relative importance, namely CYP3A (46%) and CYP1A (9%) subfamilies and CYP2C9 (16%), CYP2C19 (12%), CYP2D6 (12%), CYP2B6 (2%) and CYP2E1 (2%) isoenzymes [45]. The relative distribution is illustrated in Figure 3. CYP3A is the major isoform present in the liver and the intestine [47].

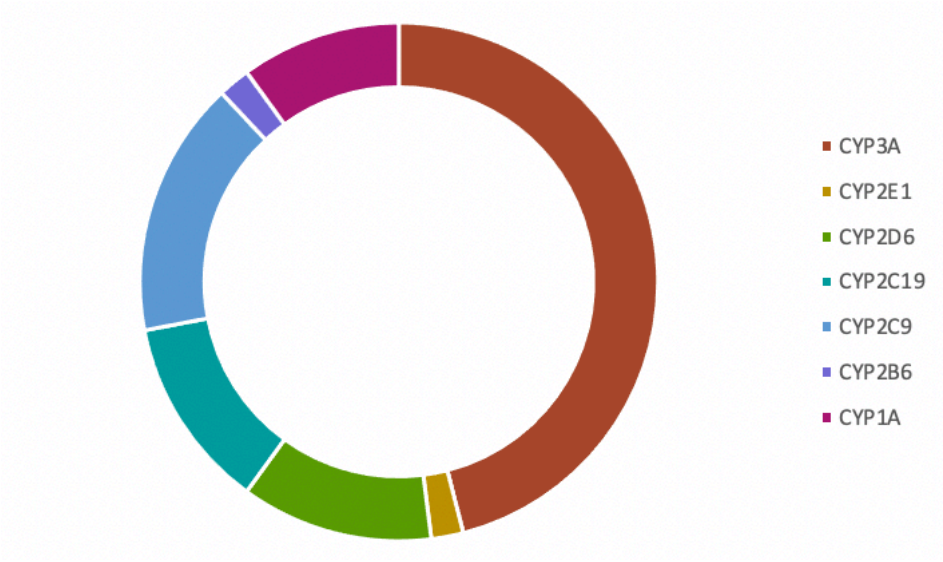

Figure 3: Relative contribution of each CYP isoenzyme in drug metabolism. Adapted from [45].

It has been reported that the inter-individual variability of CYPs activity can reach up to 50-fold for some index metabolic reactions, due to the impact of genetic, physiological, pathophysiological and environmental factors on the expression and function of CYPs [46,48].

1.3.1 Genome

Drug response is significantly influenced by the complex genetic variability of all CYPs isoforms, and can lead to drug-gene interaction (DGI) [24,42,47]. Genetic selection based on dietary and environmental poisons have been identified to be causes of differences in the distribution of CYPs alleles across ethnicities [24]. These allelic variants result from single nucleotide polymorphisms (SNPs), small deletions or insertions and copy number variants (CNVs) defined as gene deletion or duplication/amplification [12,42]. Variants are therefore classified as loss-of-function and gain-of-function variants [46]. The consequences of these changes in the structure or expression of CYP is a normal, reduced, increased or absence of activity, translating into four major phenotypes, i.e. normal metabolizer (NM), intermediate metabolizer (IM), poor metabolizer (PM) and ultra-rapid metabolizer (UM) [42]. The Veterans Health Administration recommends CYPs pharmacogenetic testing as CYPs genes may be

associated with increased risk of ADR or limited drug efficacy [49]. However, no CYPs genotyping testing was « strongly recommended » as none of the CYPs genes was considered to lead to a severe ADR and estimated probable to occur [49]. Several drug/genotype pairs were « not routinely recommended » as patients' outcomes with testing have not been demonstrated yet or because alternative investigations could provide similar clinical information, with a lesser burden on the health care system [49]. For instance, CYP2C19 and CYP2D6 polymorphisms are generally allocated to common variants, whereas 18.4% and 43.1% of CYP2C19 and CYP3A4 functional variability is caused by rare variants, respectively [43]. Moreover, ethnicity could bias these results as white Western people are mostly represented in clinical trials [15]. Women are also poorly represented, as investigators historically thought that fluctuating hormone levels make them difficult to study [17]. However, some studies now seem to recognize that ADRs affect more women [17].

CYP3A4 and CYP3A5 are the two principles isoenzymes of the CYP3A subfamilies but they cannot be distinguished due to lack of substrates specificities [38]. The distribution of CYP3A is continuous and unimodal, suggesting that it is regulated by multiple genes, resulting in a minor role of each individual genetic factors [38]. Indeed, several genetic variants have been identified but only a minority explain the five-fold constitutive variability of CYP3A4 [25,38]. The most common CYP3A4 variant is the *22 allele, associated with reduced activity and occurring in 5% of Europeans; but no genotype-based dosing guidelines have been published for CYP3A4 [12,25]. There is however a CPIC dosing guidelines for CYP3A5 pharmacogene and tacrolimus [14,25]. CYP3A5 contributes significantly to CYP3A activity and CYP3A5*3 is a well-studied non-functional variant predominant in all ethnicities, except in Africans (17%) [12,25].

In contrast, the activity of other CYPs have a polymodal distribution among the population, CYP2D6 having the highest genetic variability with 145 allelic variants identified to date, followed by CYP2C9 and CYP2C19 with 70 and 38 currently known variants, respectively [38,42].

The first genetic polymorphism identified was in 1967 on the CYP2D6 gene [24,38]. In addition to the identified variants resulting in reduced/enhanced activity or inactive enzyme, gene duplications ranging from 3 to 13 copies were described, leading to increased activity [38]. Whereas Northern Europeans are rare carriers, 29% of northeastern Africans carry gene duplications [38]. Moreover, variant frequencies vary according to ethnicities, with 10% and 1-2% of predicted PM in whites and southeast Asians respectively [12,38]. CYP2D6 high intra-individual variability can be reflected by the dextromethorphan metabolic ratio (MR), an usual

metric to assess CYP2D6 phenotype, that can vary up to 50% within healthy subjects [50]. CYP2D6 variability has a relevant clinical impact as risk of ADRs and lack of efficacy have been associated with PM and UM phenotypes [38]. A notorious example of clinical implication was discovered in Geneva by Desmeules J and colleagues in the early 1990s, with the finding that CYP2D6 had an impact on the efficacy of codeine via its bioactivation into morphine [51]. PM patients treated with codeine did not experience pain relief while an UM experience life-threatening opioid intoxication [51,52]. Guidelines concerning CYP2D6 genotype testing and prescription of codeine, tramadol, some antidepressants, as well as tamoxifen have been established [49].

CYP2C9 is the most expressed CYP2C subfamily members [25]. It has two common allelic variants (*2 and *3), frequently found in Europeans (12% and 6% respectively), but less common in the rest of ethnicities [12,25]. They are associated with CYP2C9 decreased activity, impairing the metabolism of several drugs [38]. However, the reduction of intrinsic clearance by CYP2C9*2 is dependent on the substrate and varies widely [25]. An example of the impact of CYP2C9 genetic variations can be observed with the inter-individual difference in response to warfarin, partly explained by inter-individual variations in its PK properties [2]. The genetic variations of CYP2C9 lead to serious ADRs, as warfarin has a narrow therapeutic index and it has been shown that personalized dosing guided by the genotype could improve warfarin's efficacy and safety [38,53]. However, CYP2C9 variants explain only 6-19% variability in dose requirement of warfarin, meaning that other factors are involved [25].

CYP2C19 has several inactive variants, but CYP2C19*2 and *3 are responsible for 95% of PM and their distribution is ethnic-specific with high heterogeneities [12,38]. They are associated with clinical implication as the healing rate for Helicobacter *pylori* infection is gradually higher in PM, IM and NM patients treated with a triple therapy that included a proton pump inhibitor (PPI) [54]. Moreover, a recent study showed that triple therapy was slightly more efficient when PPI dose was personalized based on CYP2C19 polymorphisms [55]. Another notorious example is clopidogrel, as loss-of-function allele (LoFA) carriers were at significant increased risk of stroke as compared to control [56]. Based on the actual knowledge, CPIC guideline recommends to avoid clopidogrel and to find an alternative antiplatelet therapy for CYP2C19 IMs and PMs [25]. In addition, CPIC dosing guidelines are available for citalopram or sertraline to avoid ADRs [14].

CYP1A2 is abundantly expressed in the liver and is involved in the metabolism of endogenous compounds and several drugs [25]. Environmental factors significantly alter the activities of CYP2C enzymes but CYP1A2 is the isoform that is the most impacted by environmental

factors [25]. Indeed, all SNPs identified only partially explain the variability that has been observed in CYP1A2 activity and it is regulated by aryl hydrocarbon receptor (AhR) pathway that is easily modulated by these factors [25]. Therefore, predicted phenotype does usually not correlate with the phenotype because it depends on the population studied [25].

CYP2B6 expression is highly variable and its expression is low in liver, as compared to other isoforms [25]. Thirty-eight variants have been identified, the most studied being CYP2B6*6, which has a reduced activity [25]. It is found in 3% of Europeans and in 16% of South Asians and is associated with efavirenz-related ADRs but the effect on clinical outcomes has not been as widely confirmed [12,25]. Evidence is emerging making thus efavirenz a possible candidate for a genotype-based dosing guideline [25]. The other known variants are either uncommon or their activity is still unclear [12,25].

1.3.2 Exposome

Genotype is associated with a predicted phenotype but in practice, misalignment between genotype and phenotype is frequently observed due to exposome interferences [49]. For example, phenotype of 13% and 47% of 114 Hungarian liver donors was underestimated and overestimated, respectively [49]. In addition, 4.0% of people were known as CYP2D6 PM based on genetic data, but 6.5% of people poorly metabolized substrates of this CYP [57]. This phenomenon is called phenoconversion, and represents a transient phenotype switch that possibly occurs when the gene variant(s) and the perpetrator have opposite effects [47]. Perpetrators are nongenetic factors altering gene expression via transcriptional factors and epigenetic mechanisms such as deoxyribonucleic acid (DNA) methylation or histones modification, and micro ribonucleic acid (miRNA) regulation [42]. Age, sex, hormone levels, environment, concomitant drugs and pathophysiological conditions such as inflammation are examples of nongenetic factors known to impact CYPs expression and activity [42]. Causes and consequences of DMET phenoconversion are discussed throughout this thesis.

Mechanistically, various transcription factors are involved in CYPs gene expression, such as AhR, pregnane nuclear receptor (PXR) and constitutive androstane receptor (CAR) [42]. CYPs isoenzymes are not sensitive to the same transcription factors, as AhR mediates the induction of CYP1 genes, PXR the induction of CYP2A6, 2B, 2C and 3A genes and CAR the induction of CYP1A, 2A6, 2B, 2C8, 2C9 and 3A4 genes [42]. Other transcription factors are also involved in CYP regulation, such as estrogen receptor element (ER8) (CYP1A), direct repeat 4 (DR4) element (CYP2A6), phenobarbital-responsive enhancer module (PBREM) (CYP2B6), xenobiotics-responsive enhancer module (XREM) (CYP2B6), glucocorticoid receptor (GR)

(CYP2C), vitamin D nuclear receptor (CYP2C), hepatocyte nuclear factor 4α (HNF4α) (CYP2C9 and CYP3A), peroxisome proliferator-activated receptor (PPAR) (CYP3A), CCAAT/enhancer-binding proteins (C/EBP) (CYP3A) [42].

1.3.2.1 Environmental factors

Gene-environment interactions involves the interaction between genes, concomitant treatments and/or lifestyle habits such as diet, alcohol consumption and smoking status [47]. Examples of the impact of environmental factors on drugs exposure are commentated in chapters 2 and 3. DDIs have been a major clinically important problem of drug treatment for decades [34,58]. DDIs are linked to CYP-catalyzed-reactions through their inhibition or induction [38,46,59]. It can happen because of the ability of a drug to selectively bind to both large active sites and distant effector or allosteric sites inside the CYPs [47]. The first CYP-dependent DDI was discovered in the early 1980s with cimetidine, and was decisive in the marketing and expansion of its competitor at the time, ranitidine [58]. It is worth noting that many approved and commonly used drugs were removed from the market because of serious ADRs triggered by coadministration with other drugs metabolized by CYPs [38]. Consequently, the Food and Drug Administration (FDA) published guidelines to evaluate *in vitro* effects of new drugs on CYPs inhibition and induction [59,60]. Candidate drugs less susceptible to variability in metabolism might be preferred over candidate drugs exhibiting a metabolism by a polymorphic CYP or subject to DDIs [61]. As a result, there has been no drug withdrawal among novel drugs due to major CYP-DDI since 2007 [58]. Many CYP inhibitors and inducers have been identified, even if not all have a clinical significance [62]. A clinical significant modulator implies that there is a relative strong affinity to a CYP at concentrations achieved in clinical situations [62]. Inhibition and induction of at least one CYP isoform are responsible for 70% and 23% of CYPs-mediated DDIs, respectively [59]. Many examples are listed in the regularly updated Geneva Table of Cytochromes P450 mediated Drug-Drug Interactions [63,64].

CYPs inhibition is ubiquitous because almost all major CYPs isoforms have been found to be inhibited by many drugs in clinical use [47]. CYPs inhibition can impair the biotransformation of drugs, leading to reduced clearance or a reduced bioactivation of a prodrug [46]. CYPs inhibition usually starts once the inhibitor is administered and its duration is often linked to the half-life of the inhibitor [46]. Mechanisms of CYPs inhibition may be competitive or non-competitive and reversible or irreversible [46]. Competitive reversible inhibition occurs with another drug that binds to the same enzyme binding site, irrespective of whether they are substrates for this CYP [46]. In non-competitive reversible inhibition, the inhibitor binds to a

site other than the active site [46]. Irreversible inhibition, or time-dependent inhibition, occurs when drugs biotransformed by CYPs interact with moieties in the active site by a covalent binding [46]. The most severe inhibition is the irreversible inhibition because the activity of the CYPs concerned will only returned to baseline after new synthesis of CYPs [47].
CYPs induction is less common but potent inducers, such as rifampicin, carbamazepine and St John's Wort, can lead to reduced exposure and to potentially weakened efficacy [47]. Induction is mainly caused by activation of transcription factors [42,47]. To a lesser extent, post-transcriptional mechanisms such as stabilization of messenger ribonucleic acid (mRNA), enzyme stabilization or inhibition of degradation protein pathways have also been described in CYPs induction [46].

Drug-drug-gene interactions (DDGIs) are classified as inhibition, induction and phenoconversion interactions [47]. DDGI is the combined effect of the genetic variant with the perpetrator drug on the metabolism pathway of a victim drug [47]. As a result, a DDGI has an effect on the magnitude of DDI in addition to have an effect on the perpetrator and/or the victim's drug concentration [47]. Therefore, this may potentiate the clinical impact of DDIs [47].

It is well acknowledged that dietary substances can modify drugs ADME through physiologic and physicochemical mechanisms, leading to food-drug interactions at clinically relevant dosing regimens [65]. Some of them can be used to improve dosing regimen and clinical outcome but they are mostly unpredictable and can have life-threatening consequences [65]. It has been shown that nutritional status and food intake influence drug metabolism and detoxification [66]. Indeed, the proportion of macronutrients and the diet content are determinant factors in the metabolism and bioavailability of drugs [66]. For example, CYP activity could be inhibited and induced by calorie-restricted diets and in diets with high intake of protein and fat, respectively [66]. Intentional or unintentional fasting decreases the activity of CYP2C9 while it increases the activity of CYP2D6 and CYP1A2, but further studies are needed to conclude on the isoform-specific impact of food intake [66]. Many drug-food interactions are described, such as CYP3A inhibition by grapefruit juice which was first described in 1989 with felodipine and could enhance systemic drug exposure by up to 14-fold depending on the substrate [67,68]. Several flavonoids, fruits chemical compounds, herbal extracts (St. John's Wort), vegetables (onions) and drinks (tea and wine) are known to modulate intestinal CYP3A4 activity [65]. In addition, consumption of cruciferous vegetables (such as broccoli and Brussels sprouts) induces CYP1A2 activity [69]. St. John's Wort is also a powerful CYP1A2, CYP2C9 and CYP2C19 inducer in the liver and small intestine [65]. Flavonoids also have an impact on several CYP isoforms [65]. Education of consumers is needed because the growing number use of over-the-counter (OTC) products with bioactive

ingredients leading to putative drug-food-interactions, while production and quality are not necessarily controlled by regulatory agencies [65].

Other life-style habits such as toxicants could also alter CYP activities. Indeed, both tobacco and marijuana smoking induce CYP1A2 and cessation requires a dose reduction of substrates [70]. Moreover, scarce data showed a potentially significant inhibition of CYP2C19 by cannabidiol (CBD) [70]. Ethanol is among the most widely used drug in the world but its effect on CYP metabolism is still unclear [71,72]. Some studies showed that hepatic CYP3A4 could be induced by ethanol while intestinal CYP3A4 might be inhibited, but with minor clinical significance [71–73]. CYP2D6 and CYP1A2 could also be inhibited by ethanol [73]. CYP2E1 is induced by ethanol, in addition of being partly responsible of its metabolism [71,72]. For instance, the increased risk of acetaminophen hepatotoxicity due to the higher toxic metabolite formation following ethanol consumption is well-characterized [71,72]. Globally, ethanol could interact with a broad variety of drugs through different mechanisms but impaired CYPs activities might be confused with the liver disease secondary to alcohol abuse [72]. Some components of red wine, such as flavonoids and other polyphenols are considered responsible for CYP3A4 and CYP1A2 inhibition [71].

Finally, environmental chemicals (ECs) are involved in the induction and inhibition of CYPs in human hepatocytes [74]. They include « chemicals contaminating natural ecosystems and specific environmental entities such as the agroecosystem, the industrial workplace, domestic living space and the environment of military deployments » [74]. For instance, pesticides are acknowledged activators of nuclear receptors such as PXR and CAR regulating the expression of CYPs [75]. Organophosphates chemicals, pyrethroids, carbamates, organochlorines insecticides and phenylureas compounds upregulate CYP3A4 and CYP2B6 expressions in the liver [75].

1.3.2.2 Physiological factors

The purpose of developmental pharmacology is to understand the impact of human growth and development on the PK and/or PD of drugs and to integrate it into clinical and therapeutic decision making [76,77]. Dr Abraham Jacobi, the father of American pediatrics, stated that « *pediatrics does not deal with miniature men and women, with reduced doses and the same class of disease in smaller bodies, but… has its own independent range and horizon* » [77]. It means that an ontogeny exists and that children and adults are not different only in terms of height and weight, but also in terms of physiological factors [78]. Therefore, PK and PD of

drugs are different in adults as compared to children, due to the maturation and development of organs and enzyme systems across the span of childhood [79].

Developmental changes in drugs PK parameters significantly contribute to the observed variations of their efficacy and safety in children [80]. For instance, it has been shown that 2.1% of pediatric admissions were due to ADRs, with a severity criteria encountered in 39% of them [81]. A good knowledge of PK developmental patterns is mandatory, as clinical trials conducted in children are lacking and less than 50% of drugs are labelled with pediatric information [78]. Children generally require a reduced dose as compared to adults, but the dose reduction would not be proportional to their weight differences [78]. Age-specific dosing requirement are currently based on the acknowledged impact of ontogeny on drugs PK [77]. However, it is a complex process, as ontogeny is not linear but rather dynamic, which leads to heterogeneity in body composition, organs function, relative size of organ systems and maturation of DME [77,81].

Ontogeny plays a role on each step of drug disposition, but the main determinant that affects drug efficacy and safety in pediatric is the difference in DME activity between adults and children [81,82]. Indeed, drug metabolism capacity is not consistent in children and can explain the toxicity in the very young [77,81]. CYP isoforms have been classified into three developmental patterns [77,81,83]:

- Class I: if it is most abundant in the fetus and decline after birth (CYP3A7)
- Class II: if its expression is relatively constant throughout gestation and in adulthood (CYP2B6 and CYP2C19)
- Class III: if its expression increases fast to reach adult levels within weeks to 1-2 years (CYP1A2, CYP3A4, CYP2C9 and CYP2D6)

A comparison of the impact of inflammation on CYPs activity between adults and pediatrics can be found in chapter 7.

Moreover, dietary differences might have an impact on CYP1A2 developmental trajectories, such as breast-fed infants who acquire CYP1A2 maturation later than formula fed infants [80]. Delayed development of CYP3A subfamily have also an impact on the absorption step, as the mRNA level of CYP3A4 in small intestine follows the same developmental trajectory as in liver (i.e. low amount at birth and rapid increase in early childhood) [83]. As a result, midazolam bioavailability is higher in premature newborns than in adults [83]. However, less is known about the developmental trajectories of other isoforms in the small intestine [83]. The large amount of data sources is a limitation for the characterization of DME in pediatrics, as they are not equally informative or cannot be extrapolated from *in vitro* data [83]. For instance, the change in mRNA levels following the developmental change in gene expression does not

necessary translate into the same changes in functional activity, or the protein content does not necessarily equate with functional *in vitro* activity [83]. Similar *in vitro* to *in vivo* data are available for CYP2D6 and for CYP2C19 and CYP2C9 at extremes of age, whereas very different patterns are found for CYP1A2 and CYP3A4 and for CYP2C19 and CYP2C9 between the ages of 0.5 and 10 years [83]. The consequence is that when differences between *in vitro* and *in vivo* data are present, *in vivo* activity is mostly greater than that observed *in vitro* and pediatric activity exceeds adults activity before returning to adult values during adolescence [83]. In addition, CYP DDIs observed in adults might not be extrapolated to children, as basal CYPs activities are not the same [81]. Consequently, developmental patterns of CYPs have to be taken into account when consequences of DDIs are studied in children [81].

Like in children, there is few prescribing information during pregnancy even though drug use is common [84]. The research article 6, which is presented later, focuses on these aspects. Drug PK is also modified in pregnant women because physiological changes occur, but limited PK trials are performed in this special population due to ethical considerations [84].
For example, CYP3A4 activity increases significantly by 35-38% during pregnancy, from 14-18 weeks of gestation until the term of pregnancy [84,85]. Studies suggest that cortisol and perhaps estradiol and progesterone circulating levels are responsible for this modification [85]. Increased CYP3A4 activity during pregnancy might lead to subtherapeutic concentrations and the need for higher dosages of CYP3A substrates to achieve adequate response [85]. For example this information is of crucial relevance for pregnant women infected with human immunodeficiency virus (HIV), as one of the goals of antiretroviral therapies is to prevent the transmission of the disease to the unborn child, knowing that many HIV drugs are CYP3A4 substrates [85]. Estradiol has also been reported to increase, by an unknown mechanism, CYP2C9 activity without altering mRNA levels *in vitro* [85]. It was confirmed by the observed increase in the unbound apparent oral clearance of phenytoin during the whole pregnancy [85]. Even if CYP2D6 is considered not inducible, pregnancy enhances its variability [84]. Indeed, NM and PM have increased and decreased activity during pregnancy, respectively [84]. CYP2D6 activity seems to increase throughout the pregnancy [85]. This change was so substantial that a switch of major route of elimination was observed for some drugs [85]. For instance, clonidine, a CYP2D6 substrate, is primarily renally eliminated in the nonpregnant population and becomes primarily metabolically eliminated during pregnancy [85]. CYP1A2 activity decreases progressively through gestation while conflicting results exist for CYP2B6 and CYP2C19 [84,85]. As oral contraceptives inhibit the expression of CYP2C19, it is expected that its activity decreases during pregnancy, because of the influence of increased levels of sexual hormones but some studies found increased clearance of proguanil [85]. A study showed that CYP2C19 NM had a lower CYP2C19 activity during pregnancy but this was not

the case for PM [84,85]. Moreover, *in vivo* and *in vitro* studies showed upregulated CYP2B6 activity probably linked to increased concentration of estradiol [84]. Nevertheless, PK parameters of efavirenz did not differ sufficiently between pregnant and nonpregnant women in a study to warrant dosage adjustment [85].

Sex-related differences have been discussed in terms of physiology and pathophysiology with clinically relevant variations in drugs safety and efficacy [86]. Among others PK factors, notable distinctions related to DME activity have been described between women and men [86]. CYP2B6, CYP2D6 and CYP3A4 activity were shown to be higher in women, CYP1A2 activity was lower, and CYP2C9 and CYP2C19 activities were similar [86]. For example, CYP3A4 activity was shown to be up to 50% higher in adult Caucasian women than men and opioids had a better efficacy but toxicity was increased in women [86]. Another example is the difference of duloxetine bioavailability between men and women, partly attributable to the inhibited CYP1A2 activity in women [87]. However, the wide intra-individual variability in duloxetine metabolism makes it impossible to adjust the dose solely on the basis of sex [87]. Many other antidepressants have a sex-related difference in terms of PK [88]. In addition, oral contraceptives are only used by women and inhibit CYP1A2 and CYP2C19 [86].

Obesity is defined as a Body Mass Index (BMI) higher than 30 kg/m^2 and a growing number of people are affected worldwide [89]. As a result, it is increasingly important to understand the impact of obesity on drugs PK and its consequences for drug dosing in an obese population [89]. Numerous obesity-related differences could influence PK in terms of physiology and pathophysiology at each step of the ADME process [89,90]. Concerning drug metabolism, 90% of obese patients have histologically proven liver injuries leading to altered enzyme activity, such as non-alcoholic fatty liver disease (NAFLD) which ranges from simple steatosis without inflammation to non-alcoholic steatohepatitis (NASH) with inflammation [89]. Patients with NAFLD have downregulated mRNA expression and corresponding CYP3A4 activity and it is proportional to the severity of the disease [89,91]. The suggested underlying mechanism is the effect of cytokines on transcription factors [91]. Whereas a trend towards increases in CYP1A2, CYP2D6, CYP2C9 and CYP2C19 activity were observed during NAFLD, body weight-normalized clearance values showed a slight decrease in CYP2C9- and CYP2C19-mediated clearance per kg of total body weight [89]. CYP2C19 decreased activity in obese patients was confirmed in two studies assessing the efficacy of the prodrug clopidogrel [92,93]. Indeed, higher BMI and CYP2C19 LoFA carriers had higher on-treatment platelet reactivity to clopidogrel, and clopidogrel/aspirin treatment was not efficient in reducing stroke recurrence in obese/overweight CYP2C19 LoFA noncarriers, as compared to normal weight LoFA noncarriers [92,93]. However, a recent systematic review concluded that obesity might

decrease the activity of CYP3A4 and CYP1A2 and increase the activity of CYP2C9, while the effect of obesity on CYP2D6 and CYP2C19 was inconclusive [66]. Further studies are needed to increase the robustness of these results.

1.3.2.3 Pathophysiological factors

The influence of disease on DMET is complex, according to the associated physiological and pathophysiological changes [91]. Animal and human studies started to report variation in the PD of drugs caused by alteration in drug metabolism during disease course, such as diabetes, cancer, hepatitis or influenza, from the early 1960s [94]. However, the interest of the pharmaceutical industry and regulatory agencies on drug-disease interactions has only grown in recent years [95]. Nowadays, the primary cause of metabolism and transport alteration has been identified by many studies as reduced DMET expression under pathophysiological conditions [94]. A range of pathophysiological factors may lead to either increased or decreased drug concentrations [96]. These factors may be present in various intensities according to disease severity, which is dynamic and thus leads to inter- and intra-individual variability in drug response [96]. Drug metabolism could be altered during disease by changes in blood flow, plasma protein concentration, DME activity and hepatic dysfunction [96]. Therefore, disease states are intrinsic factors that can lead to life-threatening ADRs or increased risk of treatment failure, especially for drugs with a narrow therapeutic index such as anticancer agents [47,94].

The kidneys and liver are the two organs responsible for the metabolism and elimination of drugs [97]. Renal diseases affect excretion through the alteration of GFR and tubular secretion and reabsorption, but also distribution, transport and biotransformation of drugs [97]. In other words, PK studies conducted in patients with renal failure have shown that non-renal clearance is decreased for several drugs [97]. One explanation is that renal dysfunction leads to pathophysiological and physiological alterations in liver [97]. The mechanism is not well characterized but one hypothesis is the accumulation of uremic toxins secondary to renal failure [36,97].
Liver disease is also associated with the accumulation of toxins, damaging cardiovascular, kidney and cerebral function but also the liver itself [36]. Liver failure may be associated with reduced production of plasma protein binding, changes in hepatic blood flow and a variable decrease in metabolizing activities, with an isoform-specific impact on CYPs activities [96]. Drug metabolism is altered during liver disease because of a disrupted hepatocytes integrity but it can also be altered during cardiac diseases [97]. Indeed, drug metabolism is affected by

hypoperfusion of the sites of drug clearance and, as a result, plasma concentrations are usually higher in patients with congestive heart failure than in healthy subjects [97].

Inflammation is another source of inter- and intra-individual variability in the PK of drugs [98]. The related clinical consequences have received less attention in the past decades than liver or kidney insufficiency or DDIs, even though inflammation is associated with many diseases [47,95]. Indeed, systemic inflammation is a complex biological process triggered in response to stimuli such as pathogens, damaged cells or irritants provided by certain lifestyles, social and environmental factors or transient injury and acute infections [98]. Once activated, immune cells trigger the inflammatory process and release various pro-inflammatory mediators such as cytokines (mainly interleukin (IL) -6, IL-1β and tumor necrosis alpha (TNF-α)), chemokines, leukotrienes or prostaglandins which, in turn, control the production of acute-phase protein (APP) such as C-reactive protein (CRP) [98]. Inflammation is now a well-known regulator of genes expression, through circulating pro-inflammatory cytokines which act as signaling molecules to modulate the activity of DMET among other PK parameters [95,98–101]. Regulation of DMETs expression and activity by inflammation occurs through induction of transcriptional inhibition, nitric oxide (NO)-dependent proteasome degradation and epigenetic modifications of genes [98]. Many studies have described a decrease in mRNA and CYPs levels with subsequent reduced CYPs activity with increased pro-inflammatory cytokine levels via pre- and post-transcriptional mechanisms [98]. However, induction was observed for certain CYPs [98]. Two comprehensive reviews of the literature on the impact of inflammation on CYP activity and PK, in adults and pediatrics, are presented in chapters 6 and 7.
Cytokines act by altering the activity of many transcriptional factors, such as nuclear factor-kappa B (NF-κB) and nuclear receptors [95]. The underlying pre-transcriptional mechanism is that cytokines activate several transcription factors by binding to their specific receptors, which in turn bind to DMET promoter regions [98]. This liaison inhibits the heterodimerization of nuclear retinoid x receptor (RXR)-α to nuclear receptors such as CAR, PXR or PPAR [98].
The interplay of nuclear receptors is responsible for the regulation of drug-processing proteins and, thus, ADME parameters of their substrates [95]. In particular, PXR and CAR are considered the most important nuclear receptors because they are xenobiotic sensors and they overlap in several aspects, including their targets (enzymes and transporters genes) [95].

Post-transcriptional mechanisms may also be responsible for the modulation of CYP activity during inflammation [98]. Some studies have shown a decrease in CYPs activity despite unchanged protein expression and others have demonstrated that cytokine-mediated downregulation of CYPs is inhibited by a NO synthase inhibitor or proteasome inhibitor [98]. Consequently, the underlying mechanism might be the increase of NO synthesis and release

through the activation of NO synthase by cytokines, reducing CYPs activity [98]. Another advanced mechanism would be the epigenetic modifications of DMET genes by inflammation, leading to DMET variation in terms of expression or activity [98]. In fact, DNA methylation in the promoters of several CYPs is inversely correlated with their level of expression [98]. In addition, miRNAs are upregulated during inflammation and lead to reduced CYPs activity [98]. It is worth noting that human inflammatory diseases are more complex than *in vitro* studies or induced-inflammation in animal models [95]. Therefore, the evaluation of their impact on the expression and activity of CYPs should be done independently [95].

1.4 P-glycoprotein

The most studied efflux transporter is the P-gp [68]. It was first described in 1976 by Juliano and Ling in Chinese Hamster Ovary (CHO) and is composed of two symmetric halves which interact as a single transporter through a decisive flexible linker region [9]. Each half comprises six transmembrane domains and a cytoplasmic adenosine triphosphate (ATP)-binding site [102].

P-gp is an ATP binding cassette (ABC) transporter (encoded by the ABCB1 gene) which mediates the transport of drugs and metabolites from the intracellular to the extracellular space by binding and translocation [68,103]. P-gp is thus located at the apical side of the barriers mediating the inside-out extrusion of different xenobiotics and is an efflux protein [9,68]. As for CYPs, it is a defense mechanism against toxins, protecting the host and his organs from the penetration of a broad spectrum of xenobiotics that leads to systemic exposure [68,104]. Besides, an overlap between CYP3A4 and ABCB1 exists concerning substrate specificity and ligands, as both are regulated by the nuclear receptor PXR [47,102].

P-gp is implicated in the absorption, distribution and elimination steps and is therefore a key determinant of PK [68,104]. By preventing several substrates from entering in the systemic circulation or in tissues and by facilitating hepatobiliary and renal drug efflux, P-gp is broadly implicated in drug efficacy and toxicity [68,75,104]. *In vitro* data could be extrapolated but it is necessary to be vigilant with animal studies as it is both species- and substrates-dependent [9]. Important pharmacotherapies such as anticancer, antivirus and immunosuppressive agents, calcium channel blockers, steroids and other drugs such as dabigatran, digoxin, fexofenadine and talinolol are P-gp substrates [99]. Many other examples are listed in the regularly updated Geneva Table of Cytochromes P450 mediated Drug-Drug Interactions [63,64]. There is a high inter- and intra-individual variability on the PK of P-gp substrates, caused by the easy modulation of P-gp expression and activity by several factors, such as

genetic polymorphism, gender, ethnicity, age, BMI but also variation over time according to diet, medication, metabolism and disease state [68,99,102,103].

1.4.1 Genome

In humans, P-gp is encoded by two genes, ABCB1 and ABCB4, but ABCB1 is responsible for encoding the drug efflux transporter while ABCB4 encodes almost exclusively for P-gp functional in phospholipid transport [9,103]. Similar to CYPs, genetic polymorphisms of P-gp have been identified [9]. The first ABCB1 variant was described in 1997 by Lankas an Umbenhauer [9]. It is worth noting that the distribution of SNPs is ethnic-dependent, with marked differences between the African and Caucasian/Asian populations [9]. Fourteen SNPs are known to induce a variation in the function of P-gp and the C3435T is the most studied [102]. However, its clinical relevance is inconsistent and the high expectations attached to this mutation have faded [102]. Other common SNPs are the C1236T and G2677T/A and these three variants have been described to influence P-gp expression [9,99,102]. These three common SNPs show strong linkage disequilibrium and the haplotypes CGC and TTT have been observed in most ethnic groups with an ethnic-dependent frequency [102]. Even though their impacts on P-gp substrates are still debated, subsequent inter-individual variability of P-gp substrates should be considered [9,99]. A trend toward higher drug concentrations in the TTT haplotype carriers cannot be fully excluded but treatment adaptation accordingly is not justified so far [102]. In addition, the influence of rare ABCB1 variants on drug bioavailability and response has not been identified yet and only few reports have described the influences of T266C, C1199A/T/C, T1985G, C2005T, T3322C or G3751A on the activity of P-gp [102]. It is thought that rare variants might have greater effects on drug PK or PD than common ones [102]. Nevertheless, almost all studies that investigated the impact of ABCB1 genetic polymorphisms on drug efficacy and safety have shown conflicting results and no consistent predictions regarding patient's drug response is possible [102]. It could be explained by the fact that there is no correlation between mRNA levels and P-gp expression and activity [104]. mRNA levels are highly variable and miRNAs could contribute to the observed inconsistency, as they inhibit the translation of mRNA into proteins [104]. A prominent role of post-transcriptional regulation exists, rather than a role of pre-transcriptional regulation of ABCB1 [104].

1.4.2 Exposome

1.4.2.1 Environmental factors

P-gp is an important mediator of DDI, as coadministration of P-gp inhibitors or inducers leads to an increase or decrease in the systemic exposure of P-gp substrates respectively [9,68,104]. PXR and/or CAR appear to be the transcription factors involved in P-gp induction, as they bind to the DR4 motif in the promoter region of the human ABCB1 gene [68]. As previously mentioned, these transcription factors are also a key regulator of CYP3A and P-gp substrates and modulators are also generally substrates and modulators of CYP3A4, or at least of another DME [68,104]. Therefore, there is a need to determine the relative importance of P-gp versus CYP3A4 modulation for each victim-perpetrator couple [100]. The FDA published guidance for the clinical evaluation of the potential transporters modulation which should be considered only if the drug in development clinically modulates CYP3A [104]. A consequence of this labeling recommendation is that a precise and large classification of CYPs substrates and perpetrators is available [104]. This is not the case for transporters and P-gp even though there have been some attempts to do so [104]. There is a knowledge gap between CYPs and P-gp, as the impact of P-gp modulation on the PK exposure has not been well-studied [104]. Indeed, many *in vitro* studies showed that several compounds are P-gp substrates, but further steps with clinical studies have not been conducted in many cases [104]. The impact of P-gp phenotypic activity on the exposure of two substrates is presented in chapter 3. However, the consequence of drug interactions involving P-gp modulation can be easily underestimated if only plasma concentration is considered [9]. Indeed, impact of P-gp modulation shows a more significant effect on tissue distribution than on plasma concentrations [9]. The classification of P-gp substrates and modulators is complex, as several factors have to be considered [104]. Selectivity and sensitivity to P-gp, site of action, elimination pathway and safety profile must be evaluated [104]. Moreover, genetic polymorphism might result in inter-individual susceptibility to gene-environment interaction [75].

P-gp induction is supposed to reduce drug bioavailability, increase renal clearance and reduce peripheral tissue distribution in intestine, kidney and peripheral tissue, respectively [104]. However, the magnitude of the decrease in P-gp substrate exposure due to induction is generally lower than for CYP3A substrates [104]. It could be explained by the fact that P-gp inducers mainly reduce bioavailability rather than elimination due to limited expression in kidney tubules, suggesting that P-gp induction occurs firstly in the intestine [104]. Therefore, the induction effect on P-gp substrates would be visible after several days when the P-gp inducers and substrate are administered simultaneously [104]. Indeed, P-gp turnover rate is

relatively fast, ranging from 5-17h, and it is estimated that P-gp level returns to baseline within a week [104]. The expected reduction of P-gp substrate exposure ranges from 20% to 67% [104]. For these reasons, short-term treatment with P-gp inducers should not lead to a significant change in the efficacy or safety profile of a P-gp substrate [104]. Rifampicin is currently known to be the most potent P-gp inducer observed, which increased by 4.2- to 3.5-fold the intestinal P-gp expression [104]. Rifampicin coadministration reduced by 19-69.5% the maximal concentration (Cmax) of P-gp substrates and increased by 21% digoxin clearance, a drug with a narrow therapeutic index [104].

Competition for drug-binding sites or blockage of ATP hydrolysis are the two mechanisms involved in P-gp inhibition [9]. It is a complex process where type of P-gp inhibition is difficult to evaluate when both perpetrator and victim drugs are coadministered [9]. This mechanism appears also to be substrate-dependent [9]. The most convincing clinical evidence of P-gp inhibition is digoxin [9]. Indeed, verapamil coadministration leads to a 40-80% increase in digoxin plasma concentration, according to dosage [9]. Another example is the P-gp inhibitor erythromycin, whose coadministration with dabigatran, increased its area under the curve (AUC) by 2-fold, irrespectively of ABCB1 variants carriers [99]. Another mechanism was described, entitled « co-operative stimulation » [9]. It was discovered because the interaction between P-gp substrates does not always follow simple kinetics and leads to P-gp activation instead of the inhibition of the P-gp-mediated transport of the substrates [9]. An overlap still exists between P-gp and CYP3A4 substrates and inhibitors and it has been suggested to use the half maximal inhibitory concentration (IC50) ratio for CYP3A4 to IC50 for P-gp as an index of the relative selectivity of a drug for inhibition mediated by P-gp or CYP3A4 to distinguish between both of them [9]. A greater ratio means a quantitatively more significant inhibition mediated by P-gp [9]. P-gp modulators examples are listed in the regularly updated Geneva Table of Cytochromes P450 mediated Drug-Drug Interactions [63,64].

Drug-food interactions were also studied with P-gp [68]. *In vitro* studies have demonstrated the inhibition of P-gp by grapefruit juice but the evidence of its clinical significance is still limited [68]. Several flavonoids, fruits chemical compounds, herbal extracts (St. John's Wort), vegetables (onions), drinks (tea and wine) and food ingredients (curcumin, piperine, ginsenosides or silymarin from milk thistle) are known to modulate intestinal P-gp activity [62,99]. Furthermore, coenzyme Q-10 is very widely consumed as a food supplement while it interferes with intestinal P-gp, leading to food-drug interaction [67]. Finally, ethanol consumption does not seem to have an impact on intestinal P-gp [67,73].

In addition to drugs, environmental pollutants and ECs can interact with transporters and modulate their activity and expression [75]. Chemical organic pesticides are the environmental

pollutants mainly considered to be involved in these interactions [75]. Humans could be broadly exposed through oral, dermal or pulmonary routes due to their extensive usage for occupational and domestic purposes and, thus wide distribution in the environment [75]. Several pesticides belonging to various classes have been observed to inhibit P-gp activity [75]. Some of them have been identified as strong P-gp inhibitors, i.e. inhibiting 50% of P-gp activity at a concentration between 100 et 250 µM [75]. Moreover, pesticides target drug-sensing receptors such as PXR and CAR, upregulating the expression of intestinal P-gp [75]. For example, chlorpyrifos, an organophosphate, markedly activates PXR and CAR, enhancing the sparse expression of P-gp [75]. However, there is only scarce experimental data for pesticides as substrates for the P-gp [75].

1.4.2.2 Physiological factors

P-gp is also concerned by ontogeny [83]. P-gp is detectable after 12 weeks of gestation in the enterocytes, reaching adults levels at birth or shortly after [83]. For instance, cyclosporine bioavailability does not change from child- to adulthood, but this information should be taken cautiously as it is also a CYP3A4 substrate that increases throughout infancy [83]. Like CYP3A4, limited data are available concerning the development of P-gp mRNA expression in the intestine of infants and children [76].

During pregnancy, renal P-gp is upregulated [84]. Indeed, a study conducted in pregnant women showed that digoxine clearance was more increased than expected by the elevation in GFR [84]. Moreover, the placenta also contains P-gp, as it acts as an anatomic barrier and protects the fetus from substances in the maternal circulation [84]. Therefore, placental P-gp expression is higher in early gestation because it is the period where the need for fetal protection is the greatest [84]. Placental P-gp expression was 45-fold higher in early pregnancy (60-90 days) as compared to the term and it seems to be regulated by human chorionic gonadotropin-beta (HCG-β) [85].

P-gp expression might also play a role in the sex-related differences observed in CYP3A4 activity [105]. Indeed, it is known that CYP3A4 activity is increased in women but discrepancy between *in vitro* and *in vivo* studies led to the revision of the initial explanation (higher protein expression in women) [105]. Initially, a plausible explanation was the presence of estrogen and progesterone in women which could modulate CYP3A4 activity, but this hypothesis was rejected because some studies showed that there is no difference in respect to the menstrual cycle phase [105]. Other hypothesis is that women have less P-gp, which leads to increased intracellular levels of a drug and thus indirectly increase CYP3A4 metabolism [105].

Some animal and human studies have shown that obesity might decrease the expression of intestinal P-gp and thus increase the bioavailability of P-gp substrates [99]. Moreover, a study conducted in Japanese obese patients showed that the G2677T/A polymorphism of ABCB1 was significantly associated with obesity and this polymorphism translates into reduced P-gp functional activity [106]. However, the effect of obesity on intestinal P-gp expression and/or function is still unclear [99].

1.4.2.3 Pathophysiological factors

The function of the barriers that express P-gp might be altered during pathophysiological conditions, such as inflammation, but its effect on DMET other than CYPs has received less attention [98,107]. As some regulatory pathways are common to both CYPs and drug transporters, the effects of cytokines on the regulation of CYPs may be relevant to transporters and especially to P-gp, due to its well-characterized overlap with CYP3A4 [95,108]. For example, NF-κB is a primary transcription factor known to regulate gene expression of many CYPs and multidrug resistance/transporters of antigen presentation (MDR/TAP) family (ABCB1) [95]. The epigenetic modification of ABCB1 gene due to inflammation might lead to variation in P-gp expression, as the modification of histone patterns inversely correlates with its level of expression [98]. Impact of inflammation on P-gp activity has been almost exclusively studied *in vitro* and in animal studies, and significant downregulation of hepatic mRNA levels of ABCB1, in a tissue- or cell-specific manner, has been reported [94,98,99,108]. However, *in-vitro* studies or induced-inflammation in animal models have a much lower level of complexity than human inflammatory diseases [95]. As a result, the impact on the expression and activity of P-gp should not be extrapolated [95]. The type of cytokines released seemed to be disease-dependent and this should be taken into consideration when evaluating an interaction between cytokines and P-gp [107]. Indeed, inflammation's impact on drug transporters is potentially dependent on the disease considered and needs further investigations [109]. For instance, P-gp seemed to be reduced or less expressed in injured hepatocytes due to hepatocellular carcinoma as compared to normal tissue [109]. In rodents, P-gp was impaired in a cholestasis model [109]. However, P-gp appeared to be upregulated in patients infected by hepatitis C virus (HCV), but downregulated in HCV-induced cirrhosis and HIV/HCV coinfection [109]. In animal models, P-gp expression seemed to be increased in NASH and primary biliary cholangitis [109]. However, results should be considered with caution as data are scarce and conflicting [109]. In addition to being disease-specific, P-gp expression and activity appear to be downregulated during inflammatory episodes with variable dose-, time- and isoform-dependent effects, as discrepancies exist between models used [108].

A decrease in protein abundance and activity is observed in patients with end-stage kidney disease [110]. This change in P-gp content leads for example to reduced transport capacity of the intestine [110]. Observations have shown that the downregulation of P-gp is caused by post-transcriptional mechanisms [110]. Indeed, creatinine clearance was inversely correlated with P-gp protein abundance and activity, suggesting the impact of a molecule present in the serum [110]. This molecule has been identified to be the uremic toxin, as for CYPs [110]. Therefore, the secretion of P-gp substrates in the intestinal lumen happens to a lesser extent during kidney failure, contributing to increased drug concentrations in addition to the reduced P-gp mediated elimination through the kidney [110]. Similar results were found during acute liver failure [110]. Indeed, the same intestinal P-gp abundance was found, but the *in vivo* function was significantly reduced [110].

1.5 Analytical methods for the *in vivo* assessment of DMET activities

Genotyping and phenotyping are two tools allowing to predict or measure the activity of DMET. The methods used to assess these in the research projects presented in chapters 3 to 5 of this thesis are presented below.

1.5.1 Genotyping

The first step is the extraction of genomic DNA from whole blood samples anticoagulated with ethylenediamine tetraacetic acid (EDTA). In our lab, we use the QIAamp® DNA blood mini kit (QIAGEN, Hilden, Germany) or the QIAsymphony® SP/AS (QIAGEN, Hilden, Germany) instrument using the QIAsymphony® DSP DNA Midi Kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol. The QIAsymphony® SP/AS instrument allows the automatization of the extraction while QIAamp DNA blood mini kit is done manually. However, it is the same principle that allows the purification of total DNA for reliable polymerase chain reaction (PCR) through different steps, i.e. the cell lysis, a purification procedure with consecutive steps and finally elution. The lysis is done via a protease or via a proteinase K, respectively. The purification with the QIAamp® DNA blood mini kit is done according to the lysate salt and pH conditions, allowing DNA optimal binding to the QIAamp® membrane. It also ensures that protein and other contaminants are not retained on the membrane following centrifugation. Concerning the QIAsymphony® DSP DNA Midi Kit, the magnetic-particle technology allows the purification of high-quality nucleic acids that are free of proteins, nucleases and other impurities, as DNA binds to magnetic particles.

The purified DNA is then quantified with the Qubit™ fluorometer (ThermoFisher Scientific, Life Technologies Holdings Pte Ltd, Singapore). Dilution and concentration steps take place to ensure that the samples are at the right normalized concentration for PCR, namely between 10 and 50 ng/µL. Our samples were normalized at a concentration of 30 ng/µL. Moreover, DNA samples must be normalized for copy number analysis according to the manufacturer's protocol [111].

The PCR instrument is a thermal cycler system coupled with a fluorescence detection system [112]. It detects the increasing amount of amplified product at any given cycle in a variable number of samples [112]. The principle of the PCR technology is to add primers (a selected polymerizing nucleotides) based on the target sequence, a master mix (containing DNA-polymerase enzymes, buffered salt and magnesium solutions) and the target DNA [113]. Appropriate thermal cycling is then applied to this mixture to obtain specific amplicons of the target sequence [113]. PCR is defined as an enzymatic-based reaction that reaches dynamic equilibrium among reactants to result in a product [113]. In quantitative PCR (qPCR), also called real-time PCR (RT-PCR), an oligonucleotide probe containing a reporter fluorescent dye (on the 5' end) and a quencher dye (on the 3' end) was also added to detect in real-time only specific amplification products [113]. When the probe is undamaged, the proximity of the reporter dye and the quencher dye suppresses the reporter fluorescence [114]. In contrast, the fluorescence increases at each PCR cycle when the probe pairs specifically to the complementary sequence during PCR, leading to the cleavage by DNA polymerase and the liberation of the reporter fluorescent dye [114]. Overall, the primers and the probe require to hybridize with the target sequence and thus, need a specific design based on the DNA sequence to produce a specific amplicon [113]. They also require the thermodynamic properties of the hybridization reaction and the template folding to reach an equilibrium to produce a final specific amplicon [113]. The preparation of a PCR system needs careful *in silico* design and extensive empirical optimization, as the thermodynamic and folding characteristics of the primers and the master mix are critical components of the assay [113].

To detect CYPs and P-gp variants in our studies, we used the TaqMan® technology, also known as 5'-nuclease reaction. It is a qPCR which is extensively used nowadays for population genetics [46,114]. TaqMan® Drug Metabolism Genotyping Assay detects potentially causative SNPs in DMET genes [111]. The general principle is that all the assays contain sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest and two TaqMan® Minor Groove Binder (MGB) probes with a non-fluorescent quencher (NFQ) [111]. Indeed, for biallelic discrimination, probe specific for each allele must be included in the PCR

[114]. One probe is labeled with VIC™ dye and the other one is labeled with FAM™ dye. They detect the Allele 1 and the Allele 2 sequence, respectively [111]. Data are then analyzed by cluster plot analysis, with FAM™ dye and VIC™ dye signals plotted on the Y- and X-axis, respectively [111]. Therefore, homozygous samples for the labeled alleles form clusters along the axis, according to the allele for which it is homozygous, while heterozygous samples cluster along the diagonal position, between homozygous clusters [111]. Indeed, a fluorescence of both signals are detected among heterozygotes [114]. The MGB probes have been developed to use shorter sequence and thus reduce the amplicon's overall size required by stabilizing the probe. Indeed, according to manufacturer, MGB group gives better quenching of the reporter by being chemically attached to the 3' end of the TaqMan® probe.
Some important DMET gene variants are triallelic SNPs, meaning that three bases occur at the same genomic location [111]. They can be studied using a pair of TaqMan® assays. Each assay contains one probe for the major SNP allele which is labeled with the same reporter dye in both assays and one probe for one of the minor alleles with the second reporter dye [111]. The pair of TaqMan® assays must be run independently on the same panel of samples. The analysis must be done together by comparing the genotype cluster position from both assays to a map of the true sample genotypes [111].

Each SNP assay can be done one by one, as we did to detect ABCB1 polymorphisms of three different SNPs. The genotyping was carried out on QuantStudio™ 12K Flex RT-PCR System (Thermo Fisher Scientific, CA, USA) with TaqMan® MGB Probe Validated SNP Genotyping Assays (Applied Biosystems, Thermo Fisher Scientific, Waltham, USA).
But nowadays, rapid, reliable, accurate and easy to perform multiplexing approaches exist [46]. They constitute high-throughput microarray-based screening methods, that allow the simultaneous detection of multiple CYPs allelic variants [46]. In our studies, we used the QuantStudio™ 12K Flex OpenArray® to assess multiple CYP genotypes simultaneously, according to the manufacturer's protocol [111,112].
The TaqMan® OpenArray™ plates involves arrays organized into 48 subarrays each composed of 64 through-holes (3072 through-holes in all) that could be designed with the number of targets and samples that fit with concerned experiments [112]. However, TaqMan® OpenArray™ plates are available, containing custom or preloaded TaqMan® GeneExpression or SNP Genotyping assays preloaded into the plate through-holes [112]. The CYPs genotype assessment in our studies was done with the pharmacogenomics Express Panel which contains 60 TaqMan® Drug Metabolism and SNP Genotyping Assay that provide coverage of essential and commonly studied markers. Each assay in the panel contains two allele-specific probes and a primer pair to discern the specific SNP target. As explained previously, the

instrument detects FAM™ and VIC™ fluorescence signals of one or two probes for gene expression [112]
TaqMan® Genotyper Software was used to analyze raw data from genotyping experiments according to the manufacturer's protocol [111].
The call rate of each SNP assay was inspected in the scatter plot and call rates for samples were also reviewed, according to the manufacturer's protocol [111]. SNP assays or samples with call rates lower than 95% have to be omitted because it indicates poor quality [111].

TaqMan® Copy Number assay Hs00010001 with ribonuclease (RNase) P as references (Thermo Fisher Scientific) was used to study the CYP2D6 gene duplication. Indeed, TaqMan® Copy Number Assay have to be run concomitantly with a TaqMan® Copy Number Reference assay, in a duplex qPCR (within a QuantStudio™ 12K Flex RT-PCR System for instance) [111,115]. Each contains two primers and a FAM™ dye label-based assay for the target of interest and the VIC® dye label-based TaqMan® Copy Number Reference Assay for RNase P [111,115]. Therefore, the copy number assay detects the target gene, and the reference assay detects a sequence that is present in two copies of the diploid genome [111,115]. In each test sample, the number of copies of the target sequence is defined by comparative quantitation using Applied Biosystems™ CopyCaller™ Software [111,115].

The star (*) allele nomenclature is a standardized allele name that identifies SNPs within drug metabolism genes [111]. It is a gene-level haplotype that often allows, when these haplotypes are associated into diplotypes, to predict the activity levels and thus, the phenotype [111]. Indeed, a star allele typically encompasses at least one causative variant [111]. In our studies, the translation of genetic pattern information from genotyping (SNPs) to pharmacogenomic gene-level star (*) nomenclature was done with the AlleleTyper™ Software and translational tables (Thermo Fisher Scientific and PharmGKB). The translational tables are found in public allele nomenclature sites and give information on DME gene star allele haplotypes, the establishing polymorphisms for these alleles and links to websites for variants having a reference SNP identifier (rs ID) [111].

1.5.2 Phenotyping

Unlike genotyping, phenotyping can detect the effect of non-genetic factors [46]. It measures « the actual *in vivo* DMET activity in an individual » and its assessment provides more information on real life enzyme/transporter activity than genetic polymorphisms [116,117]. Common phenotyping metrics are: MR, drug systemic clearance, partial clearance for a metabolic pathway or absorption rate in the case of the transporter of a probe [117].

Phenotyping metrics must be validated according to several criteria related to inter-individual variability, specificity, selectivity, independency, reproducibility, tolerability and correlation to specific PK parameters [117]. A probe drug is a compound predominantly metabolized by an individual CYP isoenzyme, that can be safely administered to humans and that has no other significant cause of variability, except for the one it will be used for as a probe [48]. Therefore, the intra-individual variability of the enzyme/transporter must be low [117]. The activity of a single CYP/transporter can be evaluated but phenotyping tests can also assess the activity of multiple CYP/transporter at the same time, through the administration of a cocktail of probe drugs [118]. Each probe that composes the cocktail is specifically metabolized by one CYP/transporter [118]. It saves time and expense, reduces intra-individual variability and provides highly selective enzyme evaluation and assessment [48]. The cocktail approach was initially developed by Breimer and Schellens in the late 1980s but Frye and colleagues reactivated it in the late 1990s with the validation of the five-drugs « Pittsburgh cocktail » [117,119,120]. Many different cocktails were developed during the last two decades, each with advantages and defaults [117,121]. In our studies, we used the « Geneva cocktail ». It is composed of caffeine, bupropion, flurbiprofen, omeprazole, dextromethorphan, midazolam and fexofenadine, to assess the activity of CYP1A2, 2B6, 2C9, 2C19, 2D6, 3A and P-gp, respectively [118]. The first Geneva cocktail was developed in 2004, and it has been improved over the years [122]. Nowadays, it is composed of low doses of caffeine 50mg, bupropion 20mg, flurbiprofen 10mg, omeprazole 10mg, dextromethorphan 10mg, midazolam 1mg and fexofenadine 25mg [122]. Low doses are used to decrease the risk of ADRs and potential DDIs between the probes but it makes the development of sensitive analytical methods mandatory to detect low concentrations [118]. In Geneva we have been measuring the drug/metabolite concentrations (MR) in blood, plasma, urine or saliva after the administration of a given probe drug [46]. The quantification of the probe substrates and their metabolite is done with validated simultaneous liquid chromatography (LC) with tandem mass spectrometry (MS/MS) [118].
LC-MS/MS is extensively used due to its high specificity compared to immunoassay and its capacity to combine the quantification of multiple analytes into one analytical run [123]. LC-MS/MS allows the consecutive following steps [123]:

- The selective separation of analytes of interest
- The electrospray ionization of these analytes
- The parents' ions selection with the correct mass-to-charge ratio (*m/z*) by the first quadrupole
- The fragmentation of the selected parents' ions into smaller fragment ions by entering a collision cell
- The fragments' ions selection by a second quadrupole with the selected *m/z*
- The detection of the fragments reaching the detector

Mass spectrometers can monitor several transitions, defined as the isolation of a product ion based on its *m/z* by the second quadrupole after fragmentation, rapidly in sequence [123]. It is worth noting that choosing the chromatography column is critical, as it needs to have the appropriate selectivity to separate the analytes of interest from others and interferences [123]. In the cocktail approach developed in our laboratory, a reverse phase is used, meaning that the most apolar molecules are eluted last [118,123]. Moreover, deuterium was used as an internal standard because it is the most common stable isotope used [118,123]. Extraction and ionization of isotopes are the same as for the compound of interest [123]. Adding isotopes early in the sample preparation protocol allows to account for any loss of sample during the extraction process and for any variation in ionization at the mass spectrometer source [123]. Moreover, our cocktail approach uses Dried Blood Spot (DBS) [118]. This sampling method requires a very low whole blood volume (10µL) from a less invasive method (small finger prick) and it avoids the use of anticoagulants and the step of plasma separation [118]. It has been demonstrated that PK profiles of all the probes used in the Geneva cocktail were comparable in DBS and plasma [124].

The comparison of MR of each probe after administration of the Geneva cocktail alone, with CYPs and P-gp inhibitors (fluvoxamine with voriconazole and quinidine) or with a CYPs and P-gp inducer (rifampicin) allowed the determination of threshold values, to categorize the patient as a PM, NM, IM or UM [124]. It has been shown to reliably predict modulation of CYPs activity after pre-treatment with CYPs modulators, and was validated under baseline CYPs activity conditions [125].

The safety of the Geneva cocktail has been confirmed in 265 healthy volunteers from three different geographic origins [126]. It has also been shown that the low-dose probes in the Geneva cocktail have no mutual DDIs, except for fexofenadine [125,127]. Indeed, the apparent clearance of fexofenadine increases by 1.7-fold and further studies are needed to assess the mechanism of the interaction [127]. However, fexofenadine has no impact on the other components of the Geneva cocktail [125].

1.6 Prediction of DMET activities using *in silico* tools

For years, PK DDIs have been experimentally evaluated to assess drugs potential risk for DDIs involving CYPs and P-gp with *in vitro* studies [59]. However, these assays are time-consuming, costly, risky and limited in their capacity to give structure-CYPs/P-gp modulating activity relationships [40,59]. These disadvantages have led to find alternative approaches [40]. *In*

silico prediction of PK is done using ligand- and structure-based approaches and drug developers are now using quantitative prediction of *in vivo* interactions from *in vitro* experiments by computational models [40,59,62]. Quantitative models might be [40]:

- Simple static
- Mechanistic static
- Mechanistic dynamic

The simple static model is the farthest from reality, frequently conducting to an overestimation of the DDI magnitude [40]. Indeed, quantification of the potential DDI is mainly based on a single constant inhibitor concentration derived from *in vitro* data, assuming that the concentration will not change over time [40]. Moreover, it is assumed that the substrate is only and fully metabolized by the liver [40].

The mechanistic static model comes closer to reality as it includes additional information such as the net effect of competitive or mechanism-based inhibition and induction [40]. It also assumes that the substrate is metabolized in the intestines, in addition to the liver [40]. However, a single constant inhibitor concentration is used and the magnitude difference between staggered and simultaneous dosing cannot be described, leading to the description of an incomplete dynamic characteristic of drug metabolism [40]. In addition, the most relevant inhibitor concentration is applied in both static models, resulting in variation of the DDI magnitude based on the inhibitor concentration [40].

The physiologically based pharmacokinetic (PBPK) model is a mechanistic dynamic model that aims to explain all drug PK characteristics and describe the variation of substrate and perpetrator concentrations in different organs over time [40]. Therefore, this model is more predictive than the static ones [40]. Indeed, the probability of DDIs occurring *in vivo* when the same *in vitro* data is analyzed is lower with PBPK models and therefore not overstated [40]. This could be explained by the use of time-variable concentrations and inter-individual variabilities such as age, sex or genetic polymorphisms in PBPK models [40]. Consequently, PBPK models are a powerful tool to assess the magnitude and range of DDIs in virtual populations [40]. So well that the FDA has gone a step further as compared to other main regulatory agencies worldwide by including its use in their guidance documents on DDIs assessment [128]. It has approved the replacement of clinical trials by PBPK models as a unique tool to estimate the PK profile and the exposure in a target tissue of a drug, based on the preclinical drug- and organ-dependent ADME data [40,129,130]. PBPK models can thus replace certain prospective studies for investigational drugs that are enzyme substrates if verified using data from clinical DDI studies with an index modulator [129]. It is one of the reasons why recent PBPK modeling development has expanded significantly [128]. PBPK modeling can also be used to predict whether an investigational drug that is an enzyme

modulator leads to DDIs, but guidance does not state if it can be used for dosing recommendations according to the predicted magnitude of the DDIs [129].

Another reason for the expansion of *in silico* approaches is their ability to evaluate large amounts of compounds at low cost, which allows for early application in the drug discovery process [59]. Preclinical development involves the increasing importance of simulation and prediction of human PK/PD through *in silico* models using *in vitro* data generated from human tissues and animal models [128,131]. The use of modeling and simulation has become an essential part of drug discovery and development in the pharmaceutical industry, influencing the selection of molecules based on their characteristics, possibly even before the physical existence of a new chemical entity (NCE) [31,128,131]. As a result, PBPK could help solve the high attrition rate problem, which usually affect 90% of all candidate compounds that pass through the development stages [59,131]. Indeed, the main cause for compounds to stop development is their performance in certain subgroups and the problem is not the effect or lack of effect of candidates in an « average » individual, but the consequence of inter-individual variability [131]. Therefore, identification of covariates in the early-stages of discovery and development is crucial and allows the production of safer and faster innovative products [31,128,131].

Essential physiological processes for drug's disposition depending on intrinsic and extrinsic factors can be described in PBPK models [40,129]. As a result, applications for PBPK models are the prediction of potential clinically relevant DDIs and preclinical/clinical PK profiles, but also [40]:

- The prediction of PK characteristics in special populations (such as pediatric, geriatric, pregnancy, obstetric and disease states)
- The prediction of PK characteristics of large molecules during drug discovery and development stages
- The determination of oral absorption characteristics (including food and/or formulation effects)
- The selection of the first-in-human dose

PBPK modeling is based on the paradigm that biological responses are better represented by the concentrations of drugs at target tissues than by external doses [132]. The use of multi-compartmental models incorporating physicochemical and physiological components in the simulation of PK data was first adapted in 1937 by Teorell [40]. PBPK model consists of several compartments, represented by different body organs/tissues and connected to each other by the systemic circulation (blood system) [40,130]. In the full PBPK model, the whole body is

considered and in the minimal PBPK model, no more than five compartments are studied, assembling organs with comparable blood flow rates to simplify the model [40]. Each compartment is described by a blood flow rate and a tissue volume and composition that are species-dependent [40,128,130]. Each tissue is considered either perfusion- (small lipophilic molecules) or permeability-rate-limited (more hydrophilic and larger molecules) [130].

Drug PK profiles are simulated with a PBPK software, which solves complex mathematical model equations and integrates algorithms [31,40]. In our lab, the designed software Simcyp® Population-based ADME Simulator is used [40]. It is a platform and database for mechanistic modeling and simulation of the processes of ADME in healthy or disease populations [131]. The possibility to integrate concomitant ADME mechanisms with a variety of compound properties in particular physiological situations is the main advantage of PBPK modeling [128]. It predicts *in vivo* PK parameters and profiles by combining experimental data, relevant physicochemical attributes of compounds and dosage form and demographic, physiological and genetic information on different patient populations [131]. Experimental data were generated during preclinical drug discovery and development, using *in vitro* enzyme and cellular systems [131]. The parameterization of PBPK models can be based on « bottom-up » (*in vitro*-derived) or « top-down » (*in vivo*-derived) data [133]. The « bottom-up » approach integrates several discrete information elements from different sources in a systematic and mechanistic framework [131]. It estimates the inter-individual variability and identifies the individuals with the most risky characteristics [131]. The « top-down » modeling approach is defined as the traditional fitting of compartmental models to observations and permits the detection of intrinsic and extrinsic factors that are responsible for inter-individual variability in drug exposure [128]. It requires investigation of covariates affecting PK data from studies and belongs to « population PK » [131]. It is therefore a fundamental part of drug development [128].

To construct a PBPK model, several input parameters have to be implemented and they are classified into three categories [40]:

- System
- Drug
- Study design

In the system category, parameters related to the physiological properties of the individual are defined, such as organ volume, mass, blood flow rate, DMET quantity, plasma protein abundance, hematocrit or genetic polymorphisms [40]. Special populations could have altered physiological properties and PBPK models thus enable to incorporate them [40].

In the drug category, parameters related to the physicochemical and ADME characteristics of drugs are defined [40]. To define the absorption process, mechanistic absorption models are required and depend on many drug-specific parameters such as molecular weight, lipophilicity, solubility and pKa values [40]. Different mechanistic absorption models have been developed across the years [40]. The distribution process of the drug in each organ uses either a perfusion or permeability rate-limited model, as mentioned above [40]. *In vivo* clearance and *in vitro*-i*n vivo* extrapolation (IVIVE) methods have been characterized by several approaches and coupled to PBPK modeling to describe whole organ clearance as clearance is the key parameter of PBPK model [40]. Indeed, it has an extensive effect on the PK behavior of the drug and IVIVE method has been developed to predict the PK profiles of humans before the first dosing [40]. Moreover, direct integration of *in vivo* clearance or back-calculation method from oral clearance to *in vitro* intrinsic clearance (retrograde approach) could be used [40]. Finally, when essential *in vitro* data and scaling factors are unknown, PK profiles can be used to estimate *in vitro* intrinsic clearance [40]. It depends on the organ considered as the application of IVIVE has been well-studied in hepatic clearance while other approaches can be used to predict *in vivo* organ clearance for non-hepatic clearance (renal or biliary excretion) [40]. GFR, amount of microsomal protein/hepatocytes per gram of liver, plasma protein, enzyme, and transporter abundances are other important system parameters [128].
In the study design category, parameters related to dose, route and frequency of administration, effect of coadministered drugs and food and formulation properties are required [40].

The final step is the validation of the developed PBPK model [40]. The procedure compares the simulated PK parameters, usually AUC and Cmax, and concentration-time profiles, by visual inspection, with the observed clinical data [40]. The mean observed/predicted ratios of the AUC and Cmax has to be comprised within the predefined success range of 2-fold [40]. The visual inspection is successful when the observed plasma concentrations are within the 5^{th} and 95^{th} percentiles of the simulated profile [40]. During this verification step, a mismatch between simulation and observation can be observed, due to uncertainties in input data and the lack of some important PK processes in the models, even if uncertainty in observed values should not be ignored [128]. Nevertheless, a parameter sensitivity analysis can identify the inputs parameters that are the most influential [130]. It allows to refine and update the PBPK models and it is called the « middle-out » approach, as it is a combination of « bottom-up » and « top-down » approaches [130]. The refinement of PBPK models is also possible according to the increasing availabilities of preclinical data, particularly *in vitro* data on ADME, and computing power [128,130]. Indeed, mismatches could occur in preclinical species and once clinical data are available, leading to the need to re-evaluate the predictive performance

of PBPK modeling throughout model development and the revision as more clinical data become accessible [128]. IVIVE enhancement has significantly participated in the recent reappearance of modern PBPK models and their refinement allows their application in drug research and development and authorization processes [130,134]. In addition, IVIVE led to the separation of the compound and system parameters, which is the paradigm for constructing generic PBPK models [134].

A concrete example of an application of PBPK model is one that has recently been developed to predict the exposure of rivaroxaban in a special population [135]. This effective PBPK model assessed the effect of drug-disease interaction (drug-drug-hepatic/renal dysfunction) concomitantly to the effect of DDIs (CYP3A and P-gp inhibitors) on rivaroxaban [135]. To simulate the population with hepatic and renal failure, the intrinsic hepatic clearance and renal clearance values in hepatic and renal dysfunction groups were applied, respectively [135]. All other parameters were the same as those in healthy subjects, and thus the PBPK model is scaled to healthy volunteers [135]. To predict the concomitant effect of DDIs with CYP3A and P-gp inhibitors on the PK profile of rivaroxaban, the fold reduction on CYP3A-mediated liver metabolism and P-gp-mediated renal excretion were subsequently incorporated into the model [135]. As expected, DDIs and drug-disease interaction demonstrated a synergistic effect of both factors on the simulation of rivaroxaban exposure [135]. Another concrete example of the development of an effective PBPK model that assessed the effect of a drug-disease interaction and a DDI on drug substrates is presented in chapter 8. It presents the development and validation of PBPK models with the help of the Simcyp® software.

1.7 References

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Chapter 2: Drug-drug interactions with direct factor Xa inhibitors: A systematic review of the literature and an analysis of VigiBase, the World Health Organization database of spontaneous safety reports.

Summary

Chapter 1 discussed that variability in drug response is due to the interplay of genetic, environmental, physiological and pathophysiological factors of individuals. Drug interactions are part of environmental factors and are of major clinical importance as they might lead to safety and efficacy issues in drug treatments. These interactions may be PK and/or PD, meaning that drug PK or PD profile may be affected and result in variations in drug concentrations or effects, respectively. All marketed drugs face this therapeutic challenge, and precision medicine could help address it. However, the pharmaceutical industry wishes to develop treatments that are suitable for the greatest number of patients for economic reasons. Consequently, numerous marketed drugs have followed the « one size fits all » development. A good knowledge of the causes and consequences of drug interactions helps to avoid standardization of drug use and to prevent over- and under-responders.

Direct oral anticoagulants (DOACs) are an example of drugs developed as a « one size fits all » treatment for patients with non-valvular atrial fibrillation (AF) or thromboembolic events. They were developed in response to the unpredictable and unsafe profile of antivitamin K drugs (AVK), resulting in a rapid substitution in clinical practice. Indeed, DOACs have several advantages but above all, they are considered to have a low potential for DDIs and food-drug interactions, and thus being at low risk of ADRs. Moreover, their dosage does not need to be individualized daily. However, they are CYP3A and P-gp substrates and **chapter 1** underscored that many intrinsic and extrinsic factors have an impact on their activity and expression. As anticoagulants, they carry an inherent risk of bleeding, making them more susceptible to PD interactions.

The **chapter 2** presents two systematic reviews (**review article 1** and **review article 2**) published in *Pharmacology Research & Perspectives* and *Personalized Medicine*, respectively. They aimed to evaluate DDIs causing ADRs with apixaban and rivaroxaban (the two most used DOACs), respectively, through a review of published data in the literature and a real-world evaluation of DDIs from the WHO global database of ICSRs. The literature search was performed for the four main DOACs and led to the identification of 160 articles. The systematic reviews included 24 articles for apixaban (15 studies and 9 case reports/series) and 59 for rivaroxaban (31 studies and 28 case reports/series). They were classified according to their mechanism of interaction. The evaluation from VigiBase retrieved 263 and 862 unique triplet combinations (apixaban or rivaroxaban – any suspected interacting drug – any ADRs) for apixaban and rivaroxaban, respectively.

Overall, these two systematic reviews highlighted that apixaban and rivaroxaban are at significant risk of DDIs contrary to what was believed at the time of marketing, especially with CYP3A/P-gp modulators or drugs that impair hemostasis. Moreover, the real-world analysis underlined that ADRs following PD interactions are more reported to pharmacovigilance entities while PK interactions seem to be inadequately detected. These DDIs can possibly be avoided with an appropriate knowledge and individualization of treatments.

My contributions to these two articles focused on the update of the literature search, the formatting of all data, the analysis of VigiBase and the discussion of the data. The first author, Dre Silvia Fernandez, did the initial literature search.

Review article 1: Drug interactions with apixaban: A systematic review of the literature and an analysis of VigiBase, the World Health Organization database of spontaneous safety reports.

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REVIEW

Drug interactions with apixaban: A systematic review of the literature and an analysis of VigiBase, the World Health Organization database of spontaneous safety reports

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Abstract

Apixaban, a direct oral anticoagulant, has emerged over the past few years because it is considered to have a low risk of drug-drug interactions compared to vitamin K antagonists. To better characterize these interactions, we systematically reviewed studies evaluating the drug-drug interactions involving apixaban and analyzed the drug-drug interactions resulting in an adverse drug reaction reported in case reports and VigiBase. We systematically searched Medline, Embase, and Google Scholar up to 20 August 2018 for articles that investigated the occurrence of an adverse drug reaction due to a potential drug interacting with apixaban. Data from VigiBase came from case reports retrieved up to the 2 January 2018, where identification of potential interactions is performed in terms of two drugs, one adverse drug reaction triplet and potential signal detection using Omega, a three-way measure of disproportionality. We identified 15 studies and 10 case reports. Studies showed significant variations in the area under the curve for apixaban and case reports highlighted an increased risk of hemorrhage or thromboembolic events due to a drug-drug interaction. From VigiBase, a total of 1617 two drugs and one adverse drug reaction triplet were analyzed. The most reported triplet were apixaban—aspirin—gastrointestinal hemorrhage. Sixty-seven percent of the drug-drug interactions reported in VigiBase were not described or understood. In the remaining 34%, the majority were pharmacodynamic drug-drug interactions. These data suggest that apixaban has significant potential for drug-drug interactions, either with CYP3A/P-gp modulators or with drugs that may impair hemostasis. The most described adverse drug reactions were adverse drug reactions related to hemorrhage or thrombosis, mostly through pharmacodynamic interactions. Pharmacokinetic drug-drug interactions seem to be poorly detected.

KEYWORDS

anticoagulants, apixaban, drug interactions, drug safety, pharmacovigilance, systemic review

1 | INTRODUCTION

Direct oral anticoagulants (DOACs) act by direct inhibition of coagulation factor II (thrombin) or factor Xa,[1,2] in contrast with heparin or vitamin K antagonists (VKAs). DOACs have emerged over the past few years from the need for a new generation of oral anticoagulants with a more predictable and safer pharmacological profile and more suitable for long-term use. They have become an alternative to VKAs, the only drugs available for long-term anticoagulation for decades.

DOACs have several advantages over other types of anticoagulants: rapid onset and offset of action, a wide therapeutic window and a predictable anticoagulant response that allows fixed doses and eliminates the need for routine monitoring. Moreover, they are considered to be at low risk of drug-drug interactions (DDIs) and food-drug interactions compared to VKAs.[2,3]

Concerning safety, DOACs have been associated with a lower risk of intracranial hemorrhage compared to VKAs and to sequential treatment with low-molecular-weight heparin (LMWH) and VKAs, regardless of their therapeutic indication.[4] There is evidence suggesting a lower mortality risk after suffering a major hemorrhage in patients under DOACs than in patients taking VKAs or LMWH-VKAs,[5,6] but conversely, DOACs are associated with a higher risk of gastrointestinal hemorrhage.[7,8]

Currently, there are five DOACs approved for use worldwide: an oral direct thrombin inhibitor, dabigatran,[9] and four oral direct factor Xa inhibitors: rivaroxaban, apixaban, edoxaban, and betrixaban.[10]

Apixaban is used for the prevention of atrial thromboembolic events in patients with nonvalvular atrial fibrillation and venous thromboembolism (VTE) recurrence and prevention in major orthopedic surgery and for the treatment of acute VTE.[11] In patients with atrial fibrillation (AF), apixaban was superior to warfarin in the prevention of stroke or systemic embolism.[12] For the treatment of acute VTA, apixaban was noninferior to enoxaparin combined with warfarin.[13] Overall, the results from the three ADVANCE trials showed a higher efficacy of apixaban than enoxaparin in the prevention of VTE after total hip or knee replacement.[14-16]

Small to modest effects in the pharmacokinetic/pharmacodynamic (PK/PD) profile of apixaban were observed in relation to sex and age, thus considered of no clinical relevance. No dose adjustments are therefore recommended for apixaban regarding sex or age alone.[11,17] Apixaban exposure increased by 30% in the low-body-weight group and decreased by 20% in the high body weight group when compared with a reference weight group. The magnitude of these changes was not considered clinically meaningful either, and no dose adjustment based on body weight alone is recommended.[18] However, a dose reduction is recommended for patients with a body weight < 60 kg and age > 80 years or serum creatinine > 1.5 mg/dL.[11] Likewise, apixaban exposure was not significantly modified by mild and moderate hepatic impairment (Child-Pugh A and B, respectively), but apixaban is contraindicated in Child-Pugh C.[11]

The half-life of apixaban is 8-15 h and it is metabolized by cytochrome P450 (CYP) 3A and is a P-glycoprotein (P-gp) substrate. Apixaban is therefore at risk of DDIs with CYP3A/P-gp inhibitors and inducers.[19,20]

The overall objective of this study was to evaluate DDIs involving apixaban by a review of the current published data available in the literature and by a real-life assessment of the data on apixaban interactions from VigiBase, the WHO (World Health Organization) global database of individual case safety reports (https://www.who-umc.org).[21]

2 | MATERIALS AND METHODS

2.1 | Literature search

To select relevant publications, we applied the eligibility criteria described in Table 1, divided into two main categories as suggested

TABLE 1 Eligibility criteria

Study characteristics	Report characteristics
Type of studies • In vitro and animal studies • Randomized controlled trials • Non-randomized studies • Observational studies (including case series and case reports) Type of participants (human studies) • Healthy subjects • Patients under DOAC therapy for any pathology Type of outcome • Effect of potential interacting drugs on PK/PD profile of DOACs • Effect of potential interacting drugs on DOACs safety profile: increase in the risk of hemorrhage or thromboembolic events • Effects of DOACs on the PK/PD profile of potential interacting drugs	Language of publication • English Type of publications • Published full-text articles • Congress abstracts Year of publication • From database inception to present (PubMed, Embase) • From 2011 to present (Google Scholar)

Abbreviations: DOAC: direct oral anticoagulant / PD: pharmacodynamic / PK: pharmacokinetic

by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[22] The literature search was conducted in two databases, namely PubMed via MEDLINE and Embase, and in Google Scholar for articles up to the 20th of August 2018.

The literature search was performed for four DOACs (apixaban, rivaroxaban, dabigatran, and edoxaban) and the search strategy was developed separately for PubMed, Embase, and Google Scholar. For PubMed, keywords/strings were (rivaroxaban OR apixaban OR dagigatran OR edoxaban) OR (DOACs OR NOAC OR « direct oral anticoagulants » OR « new oral anticoagulants » OR « direct thrombin inhibitor » OR « direct factor Xa inhibitor ») AND (drug interaction OR interaction).

In Embase, the keywords/strings used were (rivaroxaban OR apixaban OR dabigatran OR edoxaban) OR (DOACs OR NOAC OR « direct oral anticoagulants » OR « new oral anticoagulants » OR « direct thrombin inhibitor » OR « direct factor Xa inhibitor ») AND drug interaction.

Finally, in Google Scholar, the keywords rivaroxaban OR apixaban OR dabigatran OR edoxaban AND interaction OR interactions AND « case report » were applied.

The reference managing software Zotero® (version 5.0.47) removed duplicates, and two reviewers screened the title and abstract of the remaining records for potential relevance. If more than one article described a single study and each presented the same data, the most recent one was included. Articles were split into two groups: interaction studies and case reports.

The verification process was performed by reviewing the SmPC (Summary of Product Characteristics),[11] UpToDate-Lexicomp,[23] the Table of cytochromes P450 and P-gp substrates and the table of inhibitors and inducers of cytochromes P450 and P-gp (https://www.hug-ge.ch/sites/interhug/files/structures/pharmacologie_et_toxicologie_cliniques/a5_cytochromes_6_2.pdf).[24] Case reports where the DDIs was not documented or understood from a pharmacological point of view were excluded.

For interaction studies, the types of interactions assessed were PK interactions mediated by CYP3A and P-gp modulators or gastric pH modifiers and PD interactions mediated by other antithrombotic agents and nonsteroidal anti-inflammatory drugs (NSAIDs). Interactions not matching any of the previous categories were pooled into an additional category called "other drugs".

Data from these study were classified into in vitro/animal studies or phase I to phase IV human studies. Each study was reviewed and described individually. Moreover, each DDI described in an included study was compared with those described in the SmPC. This post hoc analysis allowed us to assess if some DDI were missing and if the SmPC included all data described in the literature.

For case reports, information collected (when available) was the following: patient characteristics, information on apixaban (dosage, start and end of treatment, duration of treatment) and potential interacting drugs, ADR description, and list of additional medication. A review of the list of potential interacting drugs was then performed by checking the SmPC, UpToDate-Lexicomp,the table of cytochrome P450 substrates and the table of inhibitors and inducers of cytochrome P450 and P-gp.[11,23,24]

2.2 | Analysis of data from spontaneous reports in VigiBase

To explore DDIs between apixaban and other drugs, we used spontaneous reports from VigiBase. VigiBase is maintained by the Uppsala Monitoring Centre (UMC), the WHO Collaborating Centre for International Drug Monitoring. The UMC receives reports of suspected ADRs from national centers in countries participating in the WHO Program for International Drug Monitoring (https://www.who-umc.org/vigibase/vigibase/). At the date of retrieval (02.01.2018), there were a total of 16,329,758 individual case safety reports in VigiBase for all drugs and all ADRs, and these came from 131 countries. Drugs are coded according to WHODrug and adverse drug reactions (ADR) according to MedDRA (version 20.1). The information in VigiBase comes from a variety of sources, and the probability that the suspected adverse effect is drug-related is not the same in all cases.[25]

The identification of potential DDIs from Individual Case Safety Report (ICSR) data in VigiBase is performed in terms of drug-drug-ADR (DDA) triplets. The analysis of DDA triplets to detect potential signals of DDI is performed using Omega (Ω), an observed-to expected three-way measure of disproportionate reporting developed by the UMC.[26]

Ω indicates the frequency of reporting of certain DDA triplets in the dataset compared to what is expected based on the relative reporting in the dataset. A positive Ω indicates an increased risk of the ADR when two drugs are used together compared to the sum of the individual risks when each drug is taken separately.[27] Therefore, the Ω value may increase or decrease as new reports enter VigiBase. $\Omega_{0,25}$ is used as a threshold in the screening of potential DDIs because it is the lower limit of a 95% credibility interval for Ω. Prior to analysis, the dataset was cleaned, first by removing all DDAs with $\Omega_{0,25}$ less than or equal to 0. Then, some non-relevant MedDRA preferred terms were excluded, such as “condition aggravated” because they are not real ADRs. Similarly, some non-relevant drug names were also excluded, such as “placebo” or “drug name/s under assessment for WHO-DD”. Finally, all rows with drugs reported as “concomitant” were removed from the file, therefore only drugs reported as “interacting” or “suspected” were kept. For analysis of the seriousness and the outcome, each ICSR was summarized to only one line, according to the column with the outcomes. We chose to keep the line with the worst outcome (Fatal > not recovered/not resolved > recovering/resolving > recovered/resolved with sequelae > recovered/resolved > unknown) and seriousness (death > life-threatening > caused/prolonged hospitalization > disabling/incapacitating > congenital anomaly/birth defect > other).

The search and extraction from VigiBase of ICSRs related to apixaban and DDIs was performed by the UMC on 24 April 2018 from a database freeze conducted on the 2 January 2018.

We considered the number of DDA triplets related to each MedDRA system organ class (SOC), the number of DDA triplets for apixaban and one specific ADR and the number of combinations for apixaban—one specific suspected/interacting drug in the DDA

triplet. The data for the outcome and the seriousness were extracted and their number was calculated.

We classified the DDIs as linked to the PK or PD mechanism: PK DDIs were further classified as due to absorption (PKA), distribution (PKD), metabolism (PKM), or excretion (PKE) and PD DDIs according to the direct effect at receptor function (PD1), interference with a biological or physiological control process (PD2) or additive/opposed pharmacological effect (PD3). When a DDI was verified for the two mechanisms, they were counted in both. These DDIs were classified according to SmPC, UpToDate, and PubMed. When more than one mechanism was found, all were listed.

Due to the large quantity of data extracted with the VigiBase analysis, this article focuses on apixaban only.

3 | RESULTS

3.1 | Literature

The literature search retrieved 15 interaction studies, some investigating several drugs, and 10 case reports (from nine published articles). The selection process is illustrated in the PRISMA flowchart (Figure 1) and Table 2 summarizes the interaction studies.

3.1.1 | CYP3A and P-gp inhibitors

In vitro

In an in vitro study performed by Sayani et al, apixaban did not interact with tacrolimus when combined into citrated plasma.[28]

In another in vitro study performed by Margelidon-Cozzolino et al, three PDE5 inhibitors (sildenafil, tadalafil, and vardenafil) strongly inhibited apixaban efflux by P-gp suggesting potential clinically relevant DDI.[29] The maximal inhibition was higher with vardenafil and sildenafil than with tadalafil.[29]

Phase I studies

In healthy volunteers, ketoconazole increased apixaban AUC and Cmax by 2-fold and 1.6-fold, respectively.[30] Likewise, coadministration of apixaban and diltiazem resulted in a 1.4-fold and 1.3-fold increase in apixaban AUC and Cmax, respectively.[30] In healthy volunteers, the administration of ciclosporin led to an increase of 43%

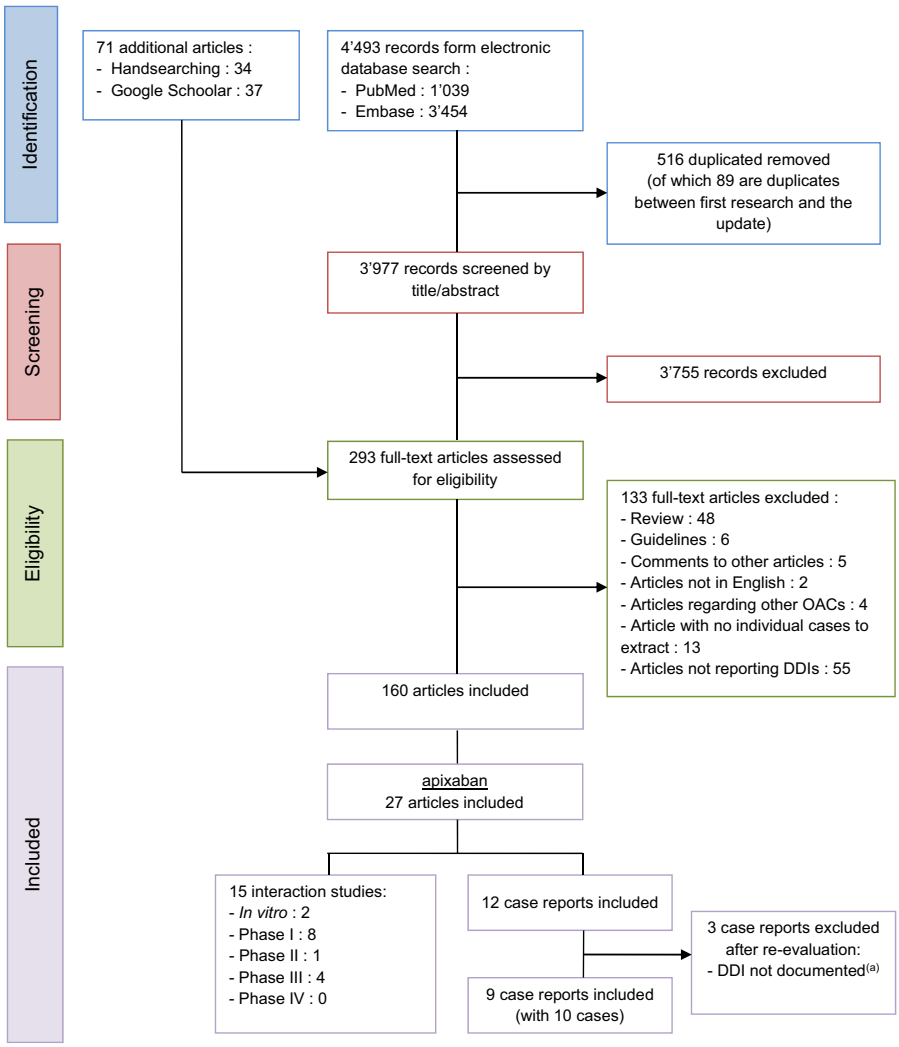

FIGURE 1 PRISMA flowchart of the apixaban studies selection process

TABLE 2 Summary of interaction studies involving apixaban

Interaction tested	Reference	Type of study	Effect observed
CYP3A4/P-gp inhibitors			
Ketoconazole	[30]	Phase I	↑ 99% AUC
Diltiazem	[30]	Phase I	↑ 40% AUC
Amiodarone	[33]	Phase III	NS effect
Tacrolimus	[28]	In vitro	No interaction
	[31]	Phase I	NS effect (↓ 22% AUC)
PDE5 (sildenafil, tadalafil, vardenafil)	[29]	In vitro	↓ efflux (97%, 74%, and 100%, respectively)
Cyclosporin	[31]	Phase I	↑ 20% AUC
Clarithromycin	[32]	Phase I	↑ 60% AUC
CYP3A4/P-gp inducers			
Rifampicin	[34]	Phase I	↓ 39% and 54% AUC (iv and oral administration, respectively)
CYP3A4/P-gp substrates			
Digoxin	[35]	Phase I	No effect
Antithrombotic agents and NSAIDs			
Enoxaparin	[36]	Phase I	↑ anti-factor Xa activity
Naproxen	[37]	Phase I	↑ 55% AUC
Aspirin	[38]	Phase II	↑ risk of bleeding
	[39]	Phase III	↑ risk of bleeding
	[40]	Phase III	↑ risk of bleeding
	[41]	Phase III	↑ risk of bleeding
Aspirin + clopidogrel	[38]	Phase II	↑ risk of bleeding
	[39]	Phase III	↑ risk of bleeding
	[40]	Phase III	↑ risk of bleeding
Gastric pH modifiers			
Famotidine	[42]	Phase I	No effect
Other drugs			
AS, CS, HA, klonopin, penicillin, TC, TA	[28]	In vitro	No effect
Atenolol	[35]	Phase I	NCR effect

Abbreviations: AS: alendronate sodium; AUC: area under the plasma concentration-time curve; CS: chondroitin sulfate; HA: hydrocodone-acetaminophen; NCR: nonclinically relevant; NS: nonsignificant; TA: tranexamic acid; TC: tramadol chlorhydrate.

and 20% in the Cmax and AUC of apixaban, respectively.[31] This did not warrant dose modification.[31] Administration of tacrolimus led to a 13% and a 22% decrease in the Cmax and the AUC of apixaban, respectively, but it did not reach statistical significance.[31] Finally, administration of clarithromycin to healthy volunteers led to an increase in the Cmax and the AUC of 30% and 60%, respectively, compared to administration of apixaban alone.[32]

Phase III studies

Flaker et al analyzed the influence of amiodarone on the outcomes of the ARISTOTLE trial, which compared apixaban and warfarin for the prevention of stroke or systemic embolism in AF patients.[33] Statistical analysis performed in their study only compared apixaban versus warfarin. Thus, there is no head-to-head comparison for each anticoagulant with or without amiodarone. Nevertheless, the observed rates for safety endpoints seem to indicate that, in the ARISTOTLE trial, there were no significant differences concerning the incidence of hemorrhagic events for apixaban with or without amiodarone (eg, the major hemorrhage rate for apixaban with amiodarone is 1.86%/year and without amiodarone is 2.18%/year).[33]

3.1.2 | CYP3A and P-gp inducers

Phase I studies

In healthy subjects, rifampicin reduced the AUC of apixaban by 54% and the Cmax by 42%.[34]

3.1.3 | CYP3A and P-gp substrates

Phase I studies

The digoxin PK profile was not affected by apixaban co-administration.[35]

3.1.4 | Other antithrombotic agents and NSAIDs

Phase I studies

A phase I study carried out by Barrett et al showed that enoxaparin did not modify the PK of apixaban. Nevertheless, enoxaparin was associated with an additive increase in the anti-factor Xa activity of apixaban.[36]

Combined administration of apixaban and naproxen increased apixaban exposure (54% increase in AUC, 61% increase in Cmax), but led to no clinically relevant prolongation of the bleeding time.[37]

Phase II studies

Apixaban was associated with a dose-dependent increase in clinically relevant hemorrhagic events during the APPRAISE trial, a phase II study in patients with recent acute coronary syndrome (ACS) receiving antiplatelet therapy (aspirin alone or with clopidogrel). This increase was more pronounced in patients receiving dual antiplatelet agents than aspirin alone with apixaban.[38]

Phase III studies

In the APPRAISE-2 trial, coadministration of apixaban with antiplatelet therapy (aspirin alone or aspirin plus clopidogrel) significantly increased major hemorrhagic events, including fatal and intracranial hemorrhages in high-risk ACS patients. This increase was not associated with a significant decrease in recurrent ischemic events, which is why the trial was terminated prematurely.[39,40]

In AF patients, the concomitant use of aspirin and apixaban or warfarin (ARISTOTLE trial) was associated with a higher hemorrhage risk in both groups. However, a similar benefit/risk profile of apixaban vs warfarin remained regardless of concomitant aspirin use.[41]

3.1.5 | Gastric pH modifiers

Phase I studies

In healthy subjects, the H_2 antagonist famotidine had no impact on apixaban's PK.[42]

3.1.6 | Other drugs

In vitro studies

No DDI was observed when apixaban was supplemented into a citrated plasma combination with the following drugs: alendronate sodium, chondroitin sulfate, hydrocodone-acetaminophen, klonopin, penicillin, tramadol chlorhydrate, and tranexamic acid.[28]

Phase I studies

A study conducted by Frost et al. established that there is no clinically relevant DDI between apixaban and atenolol. The co-administration of both drugs led to a slight decrease in apixaban exposure (15% decrease in AUC and 18% decrease in Cmax).[35]

3.2 | Case reports

Ten case reports in nine publications relating to apixaban were found in the literature.[43-51] Cases concerned mainly men except for three cases, and the age range was 43-88 years old. Apixaban indication was AF in all cases. Additional pathophysiological factors contributing to the development of the ADR were reported in several cases, the most relevant being renal impairment.

With regard to the mechanism of DDI, five cases were PK interactions, three cases were PD interaction, and two involved both PK and PD interactions. Concerning the PK interaction, two cases were treated with CYP3A and/or P-gp inhibitors and three cases were treated with P-gp and/or CYP3A inducers. For CYP3A/P-gp inhibitors, both case led to a hemorrhage, but one case involved an interaction with diltiazem and the second involved an interaction with diltiazem and amiodarone.[43,44] For the CYP3A and/or P-gp inducers, in the first case, an interaction with carbamazepine was deemed possible, but the apixaban concentrations were still lower than expected after discontinuation of carbamazepine.[47] In another case, apixaban plasma concentration increased fourfold (89 ng/mL to 361 ng/mL) after phenobarbital discontinuation.[51] In the last case of induction, the co-administration of efavirenz with apixaban led to a pulmonary embolism.[50] Two case reports described cardiac tamponade after apixaban and ibrutinib co-administration, caused by a PD interaction.[48,49] The last PD interaction involved an SSRI alone.[46] For the PK/PD interactions, one case involved a selective serotonin reuptake inhibitor (SSRI) that induced platelet dysfunction and CYP34 inhibition ([45]) and one case involved both an SSRI (platelet dysfunction) and a CYP3A/P-gp inhibitor.[46]

3.3 | VigiBase

A total of 1654 DDA triplets with positive $_{0,25}$ values were extracted from VigiBase for the DDA triplet combination apixaban—any suspected/interacting drug—any ADR. These DDA triplets came from 3137 ICSRs reported to VigiBase up to the database freeze conducted in January 2018.

After the cleaning of the dataset, 1617 DDA triplets (corresponding to 263 unique DDA triplet combinations with apixaban—one specific suspected/interacting drug—one defined ADR, each observed in several ICSRs) and 1'364 ICSRs remained for analysis.

The MedDRA SOCs most represented in the dataset were "GI disorders" (30.5%, n = 493), "investigations" (9.5%, n = 153), "respiratory, thoracic, and mediastinal disorders" (8.2%, n = 133), and "cardiac disorders" (8.0%, n = 130). The three most reported ADRs

at a MedDRA PT level in combination with apixaban and any other suspected/interacting drug were GI hemorrhage (22.7%, n = 367), decreased hemoglobin (5.1%, n = 82), and AF (4.0%, n = 64). Irrespective of the ADR, the three suspected/interacting drugs that were the most co-reported with apixaban were acetylsalicylic acid (ASA) (27,6%, n = 446), rivaroxaban (10.9%, n = 176), and clopidogrel (5.7%, n = 92). If the ADRs reported for each of those drug pairs were also considered separately, the ADR the most reported for the pair apixaban and ASA was GI hemorrhage (49.6%, n = 221), that for apixaban plus rivaroxaban was also GI hemorrhage (58.0%, n = 102) and that for the pair apixaban-clopidogrel was decreased hemoglobin (23.9%, n = 22).

The three most reported DDA triplets in the whole dataset were as follows: apixaban-ASA-GI hemorrhage (13.7%. n = 221), apixaban-rivaroxaban-GI hemorrhage (6.3%, n = 102), and apixaban-ASA-decreased hemoglobin (2.5%, n = 40).

Not all ICSRs had data regarding the seriousness and outcome. In 12.2% (n = 246) and in 4.9% (n = 67) of the ICSRs, information about the seriousness and outcome was not filled in. Figure 2 shows the results of the analysis of the data on the seriousness and the outcome reported in the ICSRs (n = 1365).

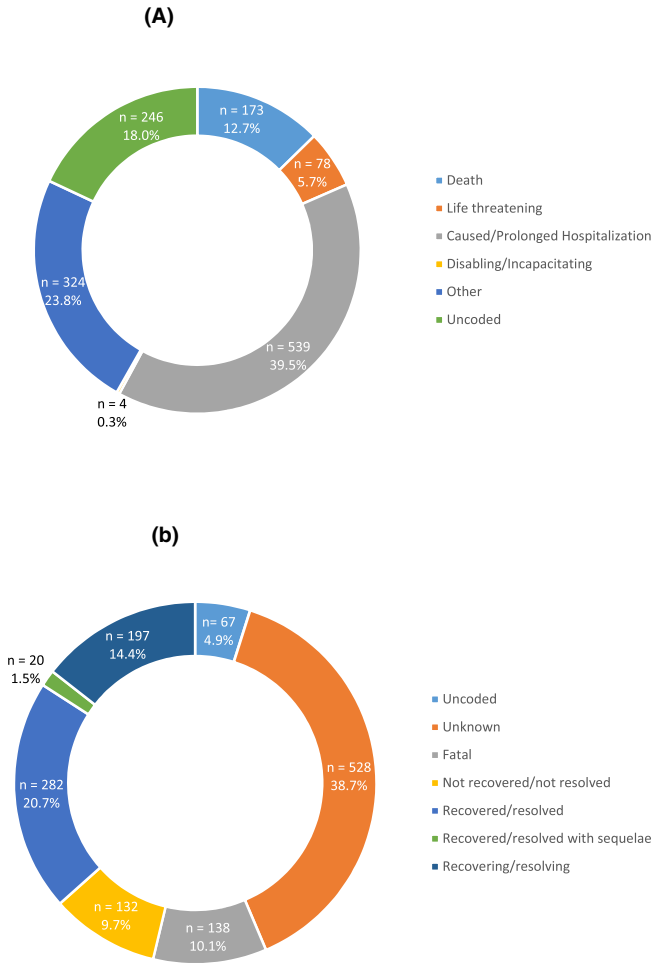

FIGURE 2 Summary of the results on the seriousness and the outcomes reported in the ICSRs extracted from VigiBase. A, Seriousness B, Outcome

Figure 2A shows the different seriousness reported and their proportions. In slightly more than a third of the ICSRs (39.5%, n = 539), the ADR was reported as caused/prolonged hospitalization. The ADR led to the patient's death in 12.7% (n = 173) of the ICSRs and was life threatening in 5.7% (n = 78). It was reported as disabling/incapacitating in only four cases (0.3%). In 23.8% (n = 324) of the cases, the seriousness was reported as "other" (those belonging to none of the other categories) (Figure 2A).

As illustrated in Figure 2B, the outcome was unknown in a large proportion of the ICSRs (38.7%. n = 528). Ten percent of the cases (10.1%, n = 138) had a fatal outcome. The patients recovered in 22.1% (n = 302) of cases (1.5%, n = 20, with sequelae and 20.7%, n = 282, without sequelae), whereas in 9.7% (n = 132) of the ICSRs the patient did not recover from the ADR. A total of 14.4% (n = 197) of the patients were deemed as recovering when the case was reported to VigiBase (Figure 2B).

Of the 263 DDA triplets reviewed, 179 DDIs were not described in the literature. For the others, a total of 12 PK DDIs, 68 PD DDIs, and 4 PK/PD DDIs were described in the literature. The most common PK DDIs was inhibition of drug metabolism, and the most common PD DDIs was additive pharmacological effect. Regarding PK DDIs, inhibitors of CYP3A and P-gp were the most reported drugs, and hemorrhagic events were the most reported ADR (Table 3). For PD DDIs, antithrombotic agents and NSAIDs were the most reported drugs, and hemorrhage was the most reported ADR. Regarding hemorrhage, the most reported site was gastro-intestinal hemorrhage (Table 3).

4 | DISCUSSION

The arrival of apixaban into routine clinical practice was a major step in anticoagulation therapy due to its alleged favorable profile, which translates into undeniable benefits for patients, especially regarding its ease of use. One of the most relevant aspects of apixaban is its theoretically low potential for interactions with other medications, food, and herbal products. However, phase IV or postmarketing studies are necessary to identify further potential DDIs, as apixaban is now used in real-world situations. To this end, we performed a literature review of published studies and case reports, together with an analysis of data reported to VigiBase. A vast majority of DDIs identified in our literature search, in both interaction studies and case reports, were DDIs with CYP3/P-gp inhibitors and other antithrombotic agents/NSAIDs. Only a few interaction studies tested the impact of CYP3A and P-gp inducers, as already pointed out in other reviews.[52,53] To verify the coverage of our literature search, we performed a post hoc comparison between our collected data and the data contained in the apixaban SmPC elaborated by the European Medicine Agency.[11] Two DDI studies described in the SmPC were not detected by our literature search, namely, a study with prasugrel and another one with the clopidogrel-ASA combination. These seem to be unpublished and not registered either in clinicaltrials.gov. Phase I

TABLE 3 Drug reported as interacting with apixaban in VigiBase with interaction mechanism and most frequently reported adverse effect

Drug B	No. of occurrence	Mechanism	Mechanism sub-classification	Most frequently reported ADRs (No. observed in parenthesis)
Acenocoumarol	1	PD	Additive pharmacological effect	Anemia (3)
Acetysalicylic acid	18	PD	Additive pharmacological effect	Gastrointestinal disorder (221)
Allopurinol	1	PD	Additive pharmacological effect	Melena (3)
Amiodarone	4	PK	Drug Metabolism	Hemorrhagic anaemia (7)
Celecoxib	1	PD	Additive pharmacological effect	Gastrointestinal hemorrhage (10)
Cilostazol	1	PD PK	- Additive pharmacological effect - Drug Metabolism	Cerebral hemorrhage (5)
Citalopram	1	PD	Additive pharmacological effect	Hematuria (3)
Clopidogrel	11	PD	Additive pharmacological effect	Hemoglobin decreased (22)
Dabigatran	1	PD	Additive pharmacological effect	Internal hemorrhage (3)
Diclofenac	2	PD	Additive pharmacological effect	Gastric ulcer hemorrhage (3) Epistaxis (3)
Diltiazem	1	PK	Drug Metabolism	Epistaxis (7)
Dronedarone	1	PK	Drug Metabolism	Transient ischemic attack (3)
Enoxaparin	3	PD	Additive pharmacological effect	Postprocedural hemorrhage (6)
Enzalutamide	1	PK	Drug Metabolism	Hematuria (3)
Fluconazole	2	PK	Drug Metabolism	- Hemorrhage intracranial (3) - Hematoma (3)
Heparin	3	PD	Additive pharmacological effect	Muscle hemorrhage (3)
Ibrutinib	7	PD	Additive pharmacological effect	Contusion (13)
Ibuprofen	4	PD	Additive pharmacological effect	Gastrointestinal hemorrhage (11)
Indometacin	1	PD	Additive pharmacological effect	Gastrointestinal disorders (4)
Loxoprofen	1	PD	Additive pharmacological effect	Gastrointestinal hemorrhage (4)
Naproxen	4 3	PD PK	- Additive pharmacological effect - Drug Metabolism	Gastrointestinal hemorrhage (9)
Phenprocoumon	2	PD	Additive pharmacological effect	Epistaxis (10)
Prednisolone	1	PD	Additive pharmacological effect	Hemorrhage subcutaneous (3)
Ranolazine	1	PK	Drug Metabolism	Hemorrhage (3)
Rivaroxaban	5	PD	Direct effect at receptor level	Gastrointestinal disorder (102)
Ticagrelor	1	PD	Additive pharmacological effect	Epistaxis (4)
Verapamil	2	PK	Drug Metabolism	Melena (3)
Warfarin	2	PD	Additive pharmacological effect	Contusion (35)

studies in healthy volunteers are not subject to mandatory data disclosure,[54,55] and their publication depends on the transparency policies of drug manufacturers. A recent study has shown a significantly lower level of transparency for phase I (healthy volunteers) studies compared to studies performed in patients.[55] Conversely, in vitro interaction studies with tacrolimus and alendronate sodium, chondroitin sulfate, hydrocodone-acetaminophen, klonopin, penicillin, tramadol chlorhydrate, and tranexamic acid identified in our review, were not mentioned in EMA SmPC because these studies showed the absence of a DDI.[11] Indeed, in vitro data are only included in the SmPC if they lead to a change in the use of the medicinal product.[56] Likewise, data from phase IV studies are only included in SmPC if they result in modification of the drug's marketing authorization.[57] Regarding in vivo data, an absence of interaction should only be mentioned in the SmPC if it is of major importance to the prescriber.[58] That may explain the absence of information on several phase I, II, and III studies showing nonsignificant or nonclinically relevant interactions. Some of the potential interacting drugs identified in the included case reports were also not mentioned in apixaban SmPC,[11] such as venlafaxine.

We also compared the ADRs reported in the case reports included in our literature search with those reported in apixaban's SmPC.[11] Hemopericardium and gluteal artery hemorrhage were identified in our case reports but were not specifically mentioned in apixaban

SmPC. However, since data from case reports alone do often not allow to establish causal relationships, further investigation would be needed to confirm these findings.[59] This is particularly true for DDIs where other factors may have also contributed to the ADR described in the case report.[60] Considering all the above, it should be underscored that our literature search has some limitations. We searched only for published articles, and thus, we did not retrieve data on unpublished interactions. Moreover, the in vitro data detected may not translate into a clinically relevant interaction in vivo.

Regarding data from VigiBase, the most co-reported suspecting/interacting drug was ASA, the most co-reported ADR was GI hemorrhage and, consequently, apixaban-ASA-GI hemorrhage was the most reported DDA triplet. DOACs have been associated with an increased risk of GI hemorrhage in multiple studies, including an evaluation of their safety profile based on data from VigiBase.[7,8] However, this phenomenon has been mainly observed with dabigatran and rivaroxaban and not with apixaban.[7,8] In the analysis from VigiBase performed by Monaco et al, apixaban was mostly associated with cerebrovascular accident,[8] an ADR not identified in our interaction dataset. Instead, our dataset included other related terms, such as ischemic stroke, transient ischemic attack or hemorrhagic cerebral infarction, although to a much lesser extent than GI hemorrhage.

Several suspected/interacting drugs were excluded from our analysis of the ICSRs, as they were not documented or understood from a pharmacological perspective as associated with DDIs with DOACs. Additionally, in many DDA triplets, the reported ADR did not seem to correlate with the drug pair, irrespective of whether the drug pair did or did not have an established DDI, such as apixaban-tamsulosin-memory impairment or apixaban-dofetilide-thirst.

We found that the proportion of PD DDIs was higher than the proportion of PK DDIs, suggesting that apixaban might be at higher-risk of interacting with drugs with the same pharmacological profile than with CYP3A4/P-gp inhibitors or inducers. However, this may be a bias, as VigiBase is a database that is dependent on spontaneous ADR reports, and healthcare professionals often know better of PD DDIs. In a study that used this same database, there were more PD DDIs (41%) than PK DDIs (25%).[61]

ADR reporting databases, such as VigiBase, have inherent limitations. The two first limitations to mention are underreporting and selective reporting. Another limitation in these databases is the lack of a denominator that allows estimating a risk. Additionally, the available dataset did not allow us to find a plausible explanation for the DDIs. They could be attributed to the heterogeneity of the data stored in VigiBase, which comes from regulatory and voluntary sources and, in some cases, may lack a proper causality assessment, since not all national pharmacovigilance centers contributing to VigiBase perform causality assessments of their ICSRs.[62] Finally, the quality and information contained in an ICSR is limited by the way this ICSR was coded into the database, with crucial data, such as the start or stop date of the drug, often missing. Information available in free text in original reports would also be important because it often contains additional relevant clinical details.[63] This approach entails a detailed case-by-case analysis of ICSRs and largely depends on the completeness of each report because it relies on fields that are not mandatory to be fulfilled for reports to be accepted in VigiBase.[64] To improve drug interaction surveillance in VigiBase, the UMC suggests the use of certain reporting patterns as indicators of DDIs in addition to a positive $\Omega_{0,25}$.[65] Other information useful in identifying suspected adverse drug interactions from ICSRs would be a plausible time course, a positive dechallenge and alternative causes of the reaction.[63] Our results have to be interpreted in this light.

5 | CONCLUSION

Our analysis shows that apixaban has significant potential for DDIs with other drugs, mostly CYP3A/P-gp inhibitors, CYP3A/P-gp inducers and drugs that may impair hemostasis, such as ASA and NSAIDs, and therefore, a significant number of DDIs with apixaban must be considered by clinicians and patients.

This review of the literature, especially the analysis of reports from VigiBase, notes that pharmacodynamic interactions that occur through the known properties of the drug and that are predictable are widely known and reported. On the other hand, the data analysis shows that the detection and reporting of pharmacokinetic interactions that occur through cytochromes or transporters are sparse because they are badly recognized.

This should motivate clinicians to stay alert on every adverse drug reaction encountered in a patient and to always consider that this adverse drug reaction could also be due to a drug-drug interaction and can be at least partly avoidable.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Review article 2: Drug-Drug Interactions Leading to Adverse Drug Reactions with Rivaroxaban: A Systematic Review of the Literature and Analysis of VigiBase.

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Review

Drug-Drug Interactions Leading to Adverse Drug Reactions with Rivaroxaban: A Systematic Review of the Literature and Analysis of VigiBase

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Abstract: Rivaroxaban has become an alternative to vitamin K antagonists, which are considered to be at higher risk of drug-drug interactions (DDI) and more difficult to use. However, DDI do occur. We systematically reviewed studies that evaluated them and analysed DDI and subsequent adverse drug reactions (ADR) reported in spontaneous reports and VigiBase. We systematically searched articles that explored DDI with rivaroxaban up to 20 August 2018 via Medline, Embase and Google Scholar. Data from VigiBase came from spontaneous reports recovered up to 2 January 2018, where Omega was used to detect signals and identify potential interactions in terms of triplets with two drugs and one ADR. We identified 31 studies and 28 case reports. Studies showed significant variation in the pharmacokinetic for rivaroxaban, and an increased risk of haemorrhage or thromboembolic events due to DDI was highlighted in case reports. From VigiBase, a total of 21,261 triplets were analysed and the most reported was rivaroxaban–aspirin–gastrointestinal haemorrhage. In VigiBase, only 34.8% of the DDI reported were described or understood, and most were pharmacodynamic DDI. These data suggest that rivaroxaban should be considered to have significant potential for DDI, especially with CYP3A/P-gp modulators or with drugs that impair haemostasis.

Keywords: rivaroxaban; drug-drug interactions; pharmacokinetic; adverse drug reaction; spontaneous reports

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1. Introduction

Unlike heparin or vitamin K antagonists (VKAs), direct oral anticoagulants (DOACs) act by direct inhibition of coagulation factor Xa or factor II (thrombin). Their pharmacological profile is deemed predictable, safe and suitable for long-term use [1]. While VKAs were the only available oral anticoagulants for more than 50 years, clinical requirements for a variety of indications in adults and the willingness to have new antithrombotic drugs on hand with an optimal balance between efficacy and risk of bleeding led to the emergence of DOACs [2]. Indeed, DOACs are considered easier to use because they have a wide therapeutic window, less interindividual variability, and higher oral bioavailability that is less impacted by food intake or bodyweight than warfarin, the reference treatment [3]. Therefore, they no longer needed to be individualised on a daily basis like VKAs, which require monitoring of the international normalised ratio (INR) [3]. However, although DOACs are less influenced by food or bodyweight, small dose adjustments are necessary for a high-dose of rivaroxaban and for low-weight patients < 60 kg taking apixaban respectively [3].

There are currently five DOACs approved for use worldwide: dabigatran, an oral direct thrombin (factor II) inhibitor [4]; rivaroxaban; apixaban; edoxaban, and; betrixaban [5], which are oral direct factor Xa inhibitors [5].

Rivaroxaban was the first oral direct factor Xa inhibitor approved, and it is used to prevent and/or treat venous thromboembolism (VTE) and prevent the occurrence of ischaemic stroke and embolism in individuals with nonvalvular atrial fibrillation (NVAF) [2]. In patients with NVAF and acute symptomatic VTE, studies have demonstrated that rivaroxaban is as effective as the standard therapy [6–8]. Moreover, rivaroxaban was superior to enoxaparin for the prevention of VTE in patients undergoing major orthopaedic surgery [9–12]. There is, therefore, no additional need for a priori monitoring of specific laboratory parameters, but anti-Xa factor could be used in specific cases where measurement is needed, for example, to confirm an overdose [13].

In addition to its ease of use and efficacy, rivaroxaban is considered to have a low risk of drug-drug interactions (DDIs), although two-thirds of rivaroxaban elimination takes place via conversion to inactive metabolites in the liver by CYP3A [3]. Rivaroxaban also carries an inherent risk of bleeding, and its coadministration with other drugs affecting haemostasis can lead to an increased risk of haemorrhage [14].

Like all DOACs, rivaroxaban has certain limitations in its use [15]. Rivaroxaban is contraindicated in women during pregnancy and lactation and in children because no data are available for these populations [16]. In addition, rivaroxaban should not be prescribed in patients with severe hepatic (Child Pugh C), renal impairment (creatinine clearance < 15 mL/min), antiphospholipid syndrome or mechanical heart valves [16]. No dose adjustments are recommended for rivaroxaban based on sex, age or bodyweight [17].

Regarding the safety profile, patients receiving rivaroxaban for any therapeutic indication have a lower risk of intracranial bleeding compared to patients receiving VKAs alone or in sequential treatment with low-molecular-weight heparins [18]. However, gastrointestinal bleeding seems to be more frequent [19,20]. Bleeding is not the only safety concern with rivaroxaban, as it has been associated with a risk of hepatotoxicity in a review that analysed data from case reports, case series and spontaneous reports [20–22]. However, in more than one-third of the drug-induced liver injuries (DILIs) observed, concomitant use of possible hepatotoxic and/or interacting drugs was also reported [21,22]. Based on these results, the authors suggested that there is a need to re-evaluate the risk of DILI associated with rivaroxaban and the importance of post-marketing pharmacovigilance to detect these potential adverse drug reactions (ADRs) [21,22].

The global aim of this study was to evaluate DDIs causing ADRs with rivaroxaban through a review of currently published data in the literature and a real-world evaluation of rivaroxaban's interaction data from VigiBase, the WHO (World Health Organization) global database of individual case safety reports (https://www.who-umc.org) [23].

2. Materials and Methods

2.1. Literature Search in Biomedical Databases

As suggested by the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) Statement, the eligibility criteria were divided into two key categories [24]. The eligibility criteria were applied to select relevant publications and are described in Table 1 [25]. The literature search was done for articles published up to 20 August 2018 in Google Scholar and in two databases, specifically, Embase and PubMed via MEDLINE. The literature search was achieved for four DOACs (rivaroxaban, apixaban [25], edoxaban and dabigatran), and the search approach was developed independently for Google Scholar, Embase and PubMed, as previously described [25]. For Google Scholar, the keywords/strings were rivaroxaban OR apixaban OR dabigatran OR edoxaban AND interaction OR interactions AND "case report". For Embase, the keywords/strings used were (rivaroxaban OR apixaban OR dabigatran OR edoxaban) OR (DOACs OR NOAC OR "direct oral anticoagulants" OR "new oral anticoagulants" OR "direct thrombin inhibitor" OR "direct factor Xa inhibitor") AND drug interaction. Finally, for PubMed, the keywords/strings used were (rivaroxaban OR apixaban OR dabigatran OR edoxaban) OR (DOACs OR NOAC OR "direct oral anticoagulants" OR "new oral anticoagulants"

OR "direct thrombin inhibitor" OR "direct factor Xa inhibitor") AND (drug interaction OR interaction).

Table 1. Eligibility criteria [25].

Study Characteristics	Report Characteristics
Type of studies • In vitro and animal studies • Randomised controlled trials • Non-randomised studies • Observational studies (including case series and case reports)	Language of publication English
Type of participants (human studies) • Healthy subjects • Patients under DOAC therapy for any pathology	Type of publications Published full-text articles and congress abstracts
Type of outcome • Effect of potential interacting drugs on PK/PD profile of DOACs • Effect of potential interacting drugs on DOACs safety profile: increase in the risk of haemorrhage or thromboembolic events • Effects of DOACs on the PK/PD profile of potential interacting drugs	Year of publication From database inception to 20 August 2018 (PubMed, Embase) and from 2011 to 20 August 2018 (Google Scholar)

DOAC: direct oral anticoagulant/PD: pharmacodynamic/PK: pharmacokinetic.

The reference managing software Zotero® (version 5.0.47) was used to remove duplicates, and the potential relevance of the remaining records was assessed by two reviewers who screened the title and abstract. If a single study was described in more than one article and each presented the same data, the most recent study was integrated. The included articles were divided into two groups, namely, interaction studies and case reports.

The mechanisms of interactions for interaction studies and case reports were checked by reviewing the Summary of Product Characteristics (SmPC) [14], UpToDate-Lexicomp [26] and the CYP450 substrates, inhibitor and inducers table (https://www.hug-ge.ch/sites/interhug/files/structures/pharmacologie_et_toxicologie_cliniques/a5_cytochromes_6_2.pdf (accessed on 20 August 2018)) [27]. Case reports that described DDIs that were not previously described or not understood from a pharmacological point of view were excluded.

As already done with apixaban in a previous article, the types of interactions assessed were PK interactions mediated by CYP3A, P-gp modulators and/or by gastric pH modifiers and PD interactions mediated by other antithrombotic agents and nonsteroidal anti-inflammatory drugs (NSAIDs) for interaction studies [25]. An additional category entitled "other drugs" pooled interactions not matching any of the previous categories. Each study was reviewed and described individually and categorised into in vitro/animal studies or phase I to phase IV human studies. Furthermore, a post hoc analysis was performed to allow us to assess if some DDIs were missing and if the SmPC included all the DDIs described in the literature.

Concerning case reports, the required information was patient characteristics, information on rivaroxaban (dosage, start and end of treatment, duration of treatment) and potential interacting drugs, adverse event descriptions and a list of additional medication when available.

2.2. Analysis of Data from Spontaneous Reports in VigiBase

Spontaneous reports from VigiBase were used to investigate DDIs between rivaroxaban and other drugs. The Uppsala Monitoring Centre (UMC) is the WHO Collaborating Centre for International Drug Monitoring and is responsible to maintain VigiBase. UMC receives reports of suspected ADRs from national centres in countries participating

in the WHO Program for International Drug Monitoring (https://www.who-umc.org/vigibase/vigibase/ (accessed on 2 January 2018)). At the date of retrieval (accessed on 2 January 2018), there were a total of 16,329,758 individual case safety reports (ICSRs) in VigiBase that came from 131 countries. Drugs are coded according to WHODrug and ADRs are coded according to MedDRA (version 20.1) [28]. The information in VigiBase comes from multiple sources, and the probability that the suspected adverse effect is drug-related is not the same in all cases [29].

Each ICSR retrieved contained drug-drug-ADR (DDA) triplets that allowed the identification of potential DDIs. The analyses to detect potential signals of DDIs were performed using Omega (Ω), an observed-to expected three-way measure of disproportionate reporting developed by the UMC [30]. When Ω is positive and two drugs are used together, an increased risk of a specific ADR occurrence is emphasised over the sum of the individual risks when these same drugs are used separately. Thus, it is an indicator of the frequency of reporting of certain DDA triplets in the dataset compared to what is expected based on the relative reporting in the dataset. The Ω value is thereby dynamic because it can change as new reports are entered in VigiBase. $\Omega_{0.25}$ is used as a threshold in the screening of potential DDIs in data from ICSRs because it is the lower limit of a 95% credibility interval for Ω. Prior to analysis, the data set was thus cleaned by removing all DDAs with $\Omega_{0.25}$ less than or equal to 0. The next step to clean the data set was to exclude some non-relevant MedDRA preferred terms, such as "stent placement", "vascular stent insertion" and "Dieulafoy's vascular malformation". Some non-relevant drug names were also excluded. Finally, all rows with drugs reported as "concomitant" were removed from the file; therefore, only drugs reported as "interacting" or "suspected" were kept. This cleaning procedure was the same as that already described in a previous publication [25].

The search and extraction of ICSRs related to rivaroxaban and DDIs from VigiBase were performed by the UMC on 24 April 2018 from a database freeze conducted on 2 January 2018 [25]. The number of DDA triplets for rivaroxaban related to each MedDRA system organ class (SOC), the number of DDA triplets for rivaroxaban and one specific ADR and the number of combinations for rivaroxaban and one specific suspected/interacting drug in the DDA triplet were studied.

According to SmPC, UpToDate and PubMed, DDIs were classified in PK and/or PD DDIs and in unknown DDIs. PK and PD DDIs were further classified into subclassifications that included absorption (PKA), distribution (PKD), metabolism (PKM) or excretion (PKE) for PK mechanisms and direct effects on receptor function (PD1), interference with a biological physiological control process (PD2) or additive/opposed pharmacological effects (PD3) for PD mechanisms. DDIs were counted in when they were verified for the two mechanisms. All mechanisms were listed when more than one was found. This article focuses on rivaroxaban only, due to the large quantity of data extracted with the VigiBase analysis. As already mentioned, similar work was done for apixaban only [25].

3. Results

3.1. Literature

The literature search retrieved 31 interaction studies, some investigating several drugs, and 28 case reports. The selection process is illustrated in the following PRISMA flowchart (Figure 1).

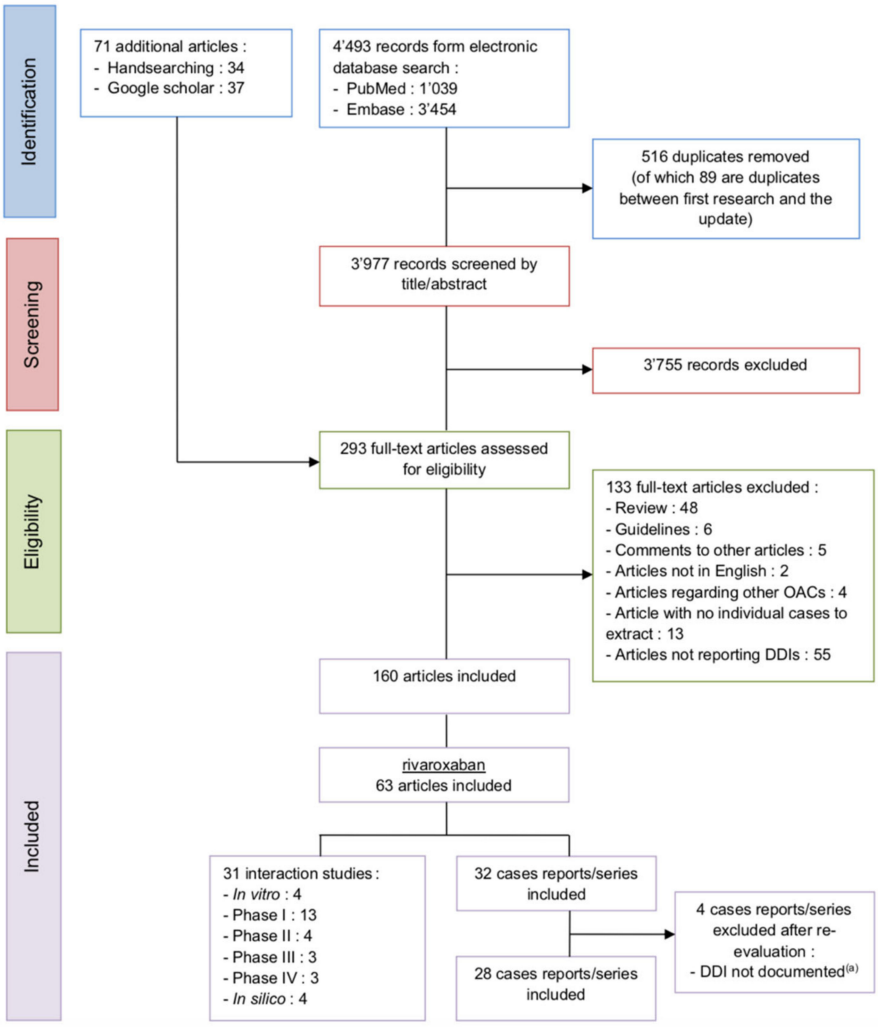

Figure 1. PRISMA flowchart of the rivaroxaban studies selection process DDI (drug-drug interaction) and OAC (oral anticoagulant), [(a)] or not understood from a pharmacological point of view.

3.1.1. CYP3A and P-gp Inhibitors

In Vitro Studies

Rivaroxaban did not show any interaction with tacrolimus when both drugs were supplemented into citrated plasma in an in vitro study [31]. In vitro, type 5 phosphodiesterase inhibitors (PDE5is), such as sildenafil, tadalafil and vardenafil, inhibited the P-gp-mediated efflux of rivaroxaban [32]. According to the authors, this could have consequences on rivaroxaban's safety, particularly in terms of bleeding risk [32].

Phase I Studies

In healthy volunteers, coadministration of ketoconazole increased the rivaroxaban AUC by 158% and the Cmax by 72% [33]. Similarly, ritonavir significantly increased the rivaroxaban AUC and Cmax by 153% and 55%, respectively [33]. Coadministration of clarithromycin, erythromycin and fluconazole with rivaroxaban significantly increased its AUC by 54%, 34% and 42%, respectively, but these moderate effects were not considered to be clinically relevant [33]. All of these coadministered drugs are CYP3A/P-gp inhibitors.

Another phase I study found a high impact of clarithromycin on rivaroxaban's PK with an AUC increase of 94% and a Cmax increase of 92%, independent of the ABCB1 genotype [34]. The effect of erythromycin on rivaroxaban exposure was also assessed in another study but this time in subjects with normal and impaired renal function. In subjects with normal renal function, coadministration with erythromycin produced an increase in

the rivaroxaban AUC and Cmax of 39% and 40%, respectively [35]. However, in subjects with mild renal impairment, the increase in the rivaroxaban AUC and Cmax when given erythromycin was 54% and 26%, respectively. Moderate renal impairment combined with erythromycin coadministration increased rivaroxaban's AUC and Cmax by 71% and 21%, respectively [35]. A study conducted in healthy volunteers demonstrated that verapamil coadministration increased the AUC of rivaroxaban. Volunteers were separated into a normal renal function group and a mild renal impairment group. The increase in the AUC was of the same extent in both groups (ratio of geometric means: 1.39 vs. 1.43, respectively) [36].

Phase II Studies

Limited data from a small phase II study in liver transplant patients (n = 9) showed an increase in rivaroxaban plasma levels in the presence of cyclosporin A (n = 5) [37]. The rivaroxaban plasma levels were within therapeutic ranges in patients treated with tacrolimus instead of cyclosporin A [37]. In a study that compared patients taking rivaroxaban (controls) and patients taking rivaroxaban and diltiazem, there was no significant difference in the incidence of major and/or clinically relevant non-major bleeding events in either group [38]. The authors suggest that although diltiazem may increase rivaroxaban exposure because of its moderate inhibition of CYP3A/P-gp, there was no evidence of an increased risk of bleeding outcomes in patients taking both drugs [38].

Phase III Studies

A study that used data from the ROCKET study assessed the risk of coadministration of non-dihydropyridine calcium channel blockers (non-DHP CCBs) with rivaroxaban or warfarin. This coadministration was not associated with a significant increase in the risk of stroke or non-central nervous system systemic embolism (p = 0.11) or major or non-major clinically relevant bleeding (p = 0.087) [39]. However, major bleeding or intracranial haemorrhage occurred more frequently in the non-DHP CCB user group (p = 0.0091 and p = 0.001, respectively) [39]. Cardiovascular death, all-cause death, myocardial infarction and all-cause hospitalisation were not significantly different between the two groups [39]. Comparison between rivaroxaban and warfarin users showed no significant difference in safety outcomes such as major or non-major clinically relevant bleeding (p = 0.14) in non-DHP CCB users [39].

Phase IV Studies

A retrospective study concluded that coadministration of amiodarone and rivaroxaban is linked to an increased risk of bleeding [40]. The study compared the number of bleeding events in patients being treated simultaneously with both drugs with patients taking rivaroxaban only [40]. Another retrospective study assessed the bleeding risk of rivaroxaban and other DOACs when it was coadministered with verapamil, diltiazem, amiodarone, dronedarone, azoles (fluconazole, ketoconazole, itraconazole, voriconazole and posaconazole), cyclosporine, erythromycin or clarithromycin [41]. The combination of rivaroxaban with amiodarone and fluconazole was associated with a significantly increased risk of major bleeding [41]. In contrast, the coadministration of rivaroxaban and erythromycin or clarithromycin decreased the risk of major bleeding but it was not statistically significant [41]. Coadministration of rivaroxaban with cyclosporin, verapamil, diltiazem, ketoconazole and itraconazole, voriconazole, posaconazole and dronedarone did not significantly change the incidence rate of major bleeding [41]. Results for erythromycin, clarithromycin, cyclosporin, verapamil, ketoconazole and dronedarone are not in line with previously cited studies. This could be because this study has strong limitations of a retrospective design and of an analysis based on the Health Insurance database system, and thus, has a lack of detailed clinical information such as liver and kidney function [41]. Moreover, this study included an Asian population that has been recognised to have a

different bleeding risk and anticoagulant therapy than the Western population [41]. Finally, rivaroxaban dosage and concomitant treatment were not considered in the model [41].

In Silico Studies

A study combined data from in vitro inhibition assays and static modelling to predict in vivo DDIs between rivaroxaban and amiodarone and dronedarone, two CYP3A/P-gp inhibitors. Thus, the study predicted an increased rivaroxaban exposure of 37% and 31%, respectively [42]. In addition, a nine percent increase in rivaroxaban exposure due to inhibition of P-gp-mediated efflux by either of the two inhibitors was estimated [42]. In a study that developed a physiologically based pharmacokinetic (PBPK) model, rivaroxaban exposure increased when DDIs with CYP3A/P-gp inhibitors (ketoconazole, ritonavir, clarithromycin) coexisted with mild or moderate hepatic dysfunction compared to hepatic dysfunction alone [43]. The simulation results revealed a synergistic effect of the addition of DDI and hepatic dysfunction, which was greatest when hepatic dysfunction was severe [43]. Another PBPK study showed that coadministration of verapamil and rivaroxaban increased rivaroxaban AUC by 1.48-fold and that coadministration of verapamil and renal insufficiency produced a synergistic increase in systemic exposure to rivaroxaban [44]. The authors suggested that subjects with mild to severe renal dysfunction who are taking verapamil should receive a reduced dose of rivaroxaban to minimise synergistic drug-drug disease interactions and prevent further bleeding risks [44]. In another PBPK model, systemic exposure to 20 mg of rivaroxaban once daily for 20 days increased when coadministered with a loading dose of amiodarone 200 mg three times a day during the last fifteen days in healthy subjects [45]. Simulations also indicated a significant 1.36-fold mean AUC increase [45]. Moreover, renal insufficiency had a synergistic effect, as the simulated mean AUC-fold change was 1.86- in patients with mild renal impairment and 1.61 in patients with moderate renal impairment where the rivaroxaban dosage was reduced to 15 mg [45].

3.1.2. CYP3A and P-gp Inducers

Phase IV Study

Coadministration of rivaroxaban with rifampicin and phenytoin was assessed and surprisingly showed an increased risk of major bleeding [41]. However, this effect was not statistically significant for rifampicin [41]. Phenytoin, as a CYP inducer, is expected to decrease rivaroxaban AUC and, therefore, the bleeding risk. The results of this phase IV study should be treated with caution due to the limitations mentioned above [41].

3.1.3. CYP3A and P-gp Substrates

Phase I Studies

No clinically relevant PK or PD interactions between rivaroxaban and the CYP3A substrate midazolam, the P-gp substrate digoxin and the CYP3A/P-gp substrate atorvastatin were observed in healthy volunteers [33,46].

Phase IV Study

The bleeding risk with rivaroxaban was assessed when coadministered with atorvastatin and digoxin and a significantly decreased risk of major bleeding was observed, while the effect of digoxin was not statistically significant [41]. In the phase I study, atorvastatin had no effect on rivaroxaban PK and this discrepancy in results can also be attributed to the limitations of the phase IV study [41].

3.1.4. Other Antithrombotic Agents and NSAIDs

In Vitro and Animal Studies

The combination of rivaroxaban with acetylsalicylic acid (ASA) and/or ticagrelor in vitro using human platelet-rich plasma and coadministration of low-dose rivaroxaban with ASA and clopidogrel in rat models of arterial thrombosis suggested that the combination of rivaroxaban with single or dual antiplatelet agents led to a synergistic increase in

their antithrombotic activity compared with anticoagulant or antiplatelet therapy alone [47]. Furthermore, the authors considered that since the low dose of rivaroxaban tested was equivalent to the trough plasma concentration after a rivaroxaban 2.5 mg twice daily dose in humans, their results can be deemed of clinical relevance [47].

Phase I Studies

No clinically relevant PK interactions were observed between rivaroxaban and enoxaparin [48] or warfarin in phase I studies [49,50]. However, some rivaroxaban PD parameters were affected, and the anti-factor Xa activity of rivaroxaban increased by 50% when coadministered with enoxaparin [48]. Regarding warfarin, an additive effect on the prolongation of the PT/INR was observed during the initial transitioning period from warfarin to rivaroxaban, although pre-treatment with warfarin did not affect rivaroxaban anti-factor Xa activity [49]. Similar results arose during the co-treatment period when switching from rivaroxaban to warfarin (higher PT and greater than additive INR values than those measured when either drug was administered alone) [50]. The combination of rivaroxaban and the commonly used NSAID naproxen significantly increased the bleeding time compared with rivaroxaban alone. On the other hand, rivaroxaban exposure was only slightly affected by coadministration of both drugs (10% increase in the rivaroxaban AUC and Cmax). The authors of the study concluded that there was no clinically relevant interaction between rivaroxaban and naproxen [51]. Moreover, the same finding was found for rivaroxaban and ASA. Rivaroxaban's bleeding time was prolonged when both drugs were coadministered as compared to rivaroxaban alone, while its PK characteristics/properties remained unchanged. Thus, the authors considered that the rivaroxaban-ASA interaction was not clinically relevant [52]. Coadministration of rivaroxaban and clopidogrel led to an additive effect on the bleeding time that was doubled when compared with the effect produced with clopidogrel alone, without affecting any other PK or PD parameters of rivaroxaban [53].

Phase II Studies

In acute coronary syndrome (ACS) patients, rivaroxaban increased the risk of bleeding events in a dose-dependent manner in both groups of patients (aspirin or aspirin and thienopyridine) compared to placebo [54]. Moreover, the absolute rate of clinically significant bleeding was higher in the group receiving dual antiplatelet therapy than in the group receiving ASA alone in addition to rivaroxaban [54]. In a study that compared the use of a low dose of rivaroxaban (2.5 mg twice daily) concomitant with either clopidogrel or ticagrelor to dual antiplatelet therapy (aspirin and either clopidogrel or ticagrelor) in patients who underwent percutaneous coronary intervention, there were no significant differences in the bleeding incidence [55]. However, in a post hoc analysis, the use of ticagrelor was associated with a significant increase in the bleeding rate ($p = 0.0006$) compared to clopidogrel [55]. As pointed out by the authors, a higher bleeding rate was found in regions where there was greater use of ticagrelor but was not associated with the randomised treatment assignment (rivaroxaban vs. aspirin) [55].

Phase III Studies

In a sub-analysis of pooled data from the RECORD programme, coadministration of NSAIDs (relative rate ratio = 1.22, CI95% = 0.99–1.50), platelet aggregation inhibitors (PAIs) and ASA (relative ratio = 1.32, CI95% = 0.85–2.05) together with rivaroxaban increased the risk of bleeding in hip or knee replacement surgery patients, although the effect was not considered significant [56]. However, the small proportion of patients using concomitant PAIs and ASA may not have been high enough to conclude on the risk of bleeding, which could explain the difference in results with other studies [56]. Regarding the increased risk of bleeding with concomitant use of NSAIDs, it was at the limit of statistical significance [56]. In the ROCKET-AF trial, more than one-third of patients were on ASA at baseline, and the concomitant use of rivaroxaban and ASA was associated with higher rates of all-cause death [57]. It is worth mentioning that the increase in all-cause death in the presence of

aspirin was more pronounced for warfarin than for rivaroxaban, enhancing the difference between the two drugs regarding outcome [57].

Phase IV Studies

In a sub-analysis of the XAMOS study, coadministration of PAIs (including ASA) increased the incidence of symptomatic thromboembolic and bleeding events in patients taking rivaroxaban and in those who followed standard thrombophylaxis for VTE prophylaxis after major orthopaedic surgery [58]. However, this finding was largely attributable to a higher incidence of symptomatic arterial thromboembolic events [58]. This could be explained by the fact that PAIs users were older and had more comorbidities affecting cardiovascular risk [58]. Additionally, concomitant use of NSAIDs was also associated with an increased risk of bleeding, while it had no influence on the rate of symptomatic thromboembolic events [58].

3.1.5. Gastric pH Modifiers

Phase I Studies

Ranitidine, a H_2 antagonist, has no significant impact on the PK/PD of rivaroxaban [59]. Similarly, the proton pump inhibitor (PPI) omeprazole showed no clinically relevant PK or PD interactions with rivaroxaban [60].

3.1.6. Other Drugs

In Vitro Studies

Irinotecan is metabolised by esterases to its active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin), which is later detoxified via glucuronidation to form SN-38G. In human liver microsomes, rivaroxaban displayed dose-dependent inhibition of SN-38 glucuronidation, which may increase SN-38 toxicity [61]. These findings suggest a potential interaction between rivaroxaban and irinotecan [61]. The combination of rivaroxaban with drugs such as alendronate sodium, chondroitin sulfate, hydrocodone-acetaminophen, clonazepam, penicillin, tramadol and tranexamic acid did not exhibit any interactions [31].

Results are summarised in Table 2.

Table 2. Summary of DDIs involving rivaroxaban.

Interactions Tested	Drugs Tested	References	Type of Study	Effect Observed
CYP3A/P-gp inhibitors	Amiodarone	[40]	Phase IV	↑ risk of bleeding
		[41]	Phase IV	↑ risk of major bleeding
		[42]	In silico	37% ↑ AUC
		[45]	In silico	×1.36 AUC
	Dronedarone	[41]	Phase IV	No increased risk of major bleeding
		[42]	In silico	31% ↑ AUC
	Clarithromycin	[33]	Phase I	54% ↑ AUC
		[34]	Phase I	94% ↑ AUC
		[41]	Phase IV	No increased risk of major bleeding
		[43]	In silico	×1.3 AUC
	Cyclosporine A	[37]	Phase II	102.6% ↑plasma levels
		[41]	Phase IV	No increased risk of major bleeding
	Erythromycin	[33]	Phase I	34% ↑ AUC
		[35]	Phase I	39% ↑ AUC
		[41]	Phase IV	N
	Diltiazem	[38]	Phase II	No significant increased risk of bleeding or thromboembolic event
		[41]	Phase IV	No increased risk of major bleeding

Table 2. *Cont.*

Interactions Tested	Drugs Tested	References	Type of Study	Effect Observed
	Fluconazole	[33]	Phase I	42% ↑ AUC
		[41]	Phase IV	↑ risk of major bleeding
	Itraconazole	[41]	Phase IV	No increased risk of major bleeding
	Ketoconazole	[33]	Phase I	158% ↑ AUC
		[41]	Phase IV	No increased risk of major bleeding
		[43]	In silico	×2.3 AUC
	Non-DHP CCB	[39]	Phase III	No significant increased risk of thromboembolic event or clinically relevant bleeding ↑ risk of major bleeding or intracranial haemorrhage
	PDE5is	[32]	In vitro	↑ risk of bleeding
	Ritonavir	[33]	Phase I	153% ↑ AUC
		[43]	In silico	×2.2 AUC
	Tacrolimus	[62]	In vitro	No interaction
		[37]	Phase II	Plasma levels within therapeutic range (internal reference, 7–65 ng/mL)
	Verapamil	[36]	Phase I	38–41% ↑ AUC
		[41]	Phase IV	No increased risk of major bleeding
		[44]	In silico	48% ↑ AUC
	Voriconazole	[41]	Phase IV	No increased risk of major bleeding
CYP3A/P-gp inducers	Phenytoin	[41]	Phase IV	↑ risk of major bleeding
	Rifampicin	[41]	Phase IV	No increased risk of major bleeding
CYP3A/P-gp substrates	Atorvastatin	[41]	Phase IV	↓ risk of major bleeding
		[46]	Phase I	NCR effect
	Digoxin	[41]	Phase IV	No increased risk of major bleeding
		[46]	Phase I	NCR effect
	Midazolam	[33]	Phase I	NCR effect
Antithrombotic agents and NSAIDs	Aspirin	[47]	In vitro	↑ antithrombotic activity
		[52]	Phase I	↑ bleeding time
		[54]	Phase II	↑ risk of bleeding
		[55]	Phase II	No significant difference in the bleeding incidence
		[56]	Phase III	No increase in the risk of bleeding
		[57]	Phase III	↑ risk of all-cause death
		[58]	Phase IV	↑ risk of bleeding and ↑ risk of symptomatic thromboembolism
	Aspirin + clopidogrel	[47]	In vitro	↑ antithrombotic activity
	Aspirin + ticagrelor	[47]	In vitro	↑ antithrombotic activity
	Aspirin + thienopyridine	[54]	Phase II	↑ risk of bleeding
	Clopidogrel	[53]	Phase I	↑ Bleeding time
		[55]	Phase II	Significant decrease in the bleeding rate as compared to ticagrelor
	Enoxaparin	[48]	Phase I	50% ↑ anti-factor Xa activity

Table 2. *Cont.*

Interactions Tested	Drugs Tested	References	Type of Study	Effect Observed
	Naproxen	[51]	Phase I	↑ bleeding time and 10% ↑ AUC
	NSAIDs	[56]	Phase III	No increased risk of bleeding (but limit of significance)
		[58]	Phase IV	↑ risk of bleeding
	Platelet aggregation inhibitor	[56]	Phase III	No increased risk of bleeding
		[58]	Phase IV	↑ risk of bleeding and ↑ risk of symptomatic thromboembolism
	Ticagrelor	[47]	In vitro	↑ antithrombotic activity
	Warfarin	[49]	Phase I	↑ PT/INR
		[50]	Phase I	↑ PT/INR
Gastric pH modifiers	Omeprazole	[60]	Phase I	NCR effect
	Ranitidine	[59]	Phase I	NCR effect
Other drugs	Irinotecan	[61]	In vitro	Inhibition of irinotecan active metabolite glucuronidation
	AS, CS, HA, klonopin, penicillin, TC, TA	[62]	In vitro	No effect

AS: alendronate sodium, AUC: area under the plasma concentration-time curve, CS: chondroitin sulphate, HA: hydrocodone-acetaminophen, INR: international normalised ratio, NCR: non-clinically relevant, PT: prothrombin time, TA: tranexamic acid, TC: tramadol chlorhydrate.

3.2. Case Series or Reports

Twenty-eight case reports were found in the literature. Eleven cases were female, with an overall age range of 29–90 years. Among them, four patients died. The rivaroxaban indication was mainly AF (n = 16) but also VTE prevention after orthopaedic surgery (n = 7), recurrent VTE prevention (n = 2), VTE treatment (n = 1), transient ischaemic attack (n = 1) and unknown (n = 1). Renal impairment (n = 7) was the most relevant pathophysiological factor contributing to the development of ADRs. Concerning the mechanism of interaction, PK DDIs were involved in seventeen cases [63–79], PD DDIs in eight cases [80–87] and PK/PD DDIs in three cases [88–90]. Bleeding (n = 18) and thromboembolic events (n = 7) were the two main ADRs described in these case reports. In two other cases, the coagulation parameters were abnormal, and the anti-Factor Xa peak remained under the reference value, but this had no consequences [78,89]. In one case, rivaroxaban induced hepatic encephalopathy that led to death [90]. In the cases describing thromboembolic events or lack of efficacy measured with laboratory tests (coagulation parameters or anti-Factor Xa), the involved comedications were CYP3A and/or P-gp inducers, namely, rifampicin [68,73], nevirapine [72] and antiepileptic drugs, such as carbamazepine [64,66,77], oxcarbamazepine [65] or phenytoin [78]. PK DDIs with CYP3A and/or P-gp inhibitors led to bleeding events in all cases. The PD DDIs involved coadministration of alirocumab [80] and antiplatelet aggregation drugs such as clopidogrel [80,86] or aspirin [87], warfarin [81,85], NSAIDs [83,84] and cocaine [82].

3.3. VigiBase

A total of 21,261 DDAs with positive $\Omega_{0.25}$ values were extracted from VigiBase for the DDA combination of rivaroxaban with any suspected/interacting drug and any ADR. Those DDAs came from 18,928 ICSRs reported to VigiBase up to the database freeze in January 2018. After cleaning the datasets, 21,109 DDAs (corresponding to 862 unique DDA combinations of rivaroxaban with one specific suspected/interacting drug and one defined ADR, each observed in a certain number of ICSRs). In the dataset, the most represented MedDRA SOCs were GI disorders (n = 12,307, 58.3%), renal and urinary

disorders (n = 1994, 9.4%) and vascular disorders (n = 1533, 7.3%). For the ADRs, the three most reported in combination with rivaroxaban and any other suspected/interacting drug were GI haemorrhage (n = 7182, 34.0%), upper GI haemorrhage (n = 1619, 7.7%) and rectal haemorrhage (n = 1355, 6.4%). Regardless of the ADR, acetylsalicylic acid (ASA) (n = 12,725, 60.3%), clopidogrel (n = 2464, 11.7%) and warfarin (n = 1110, 5.3%) were the three suspected/interacting drugs most co-reported with rivaroxaban. If the ADRs reported for each of those drug pairs were also considered, the most reported ADR was GI haemorrhage, with incidence rates of 38.0% (n = 4838), 40.9% (n = 1009) and 36.6% (n = 406), respectively.

The three most reported DDAs in the whole dataset were:

- rivaroxaban–ASA–GI haemorrhage (n = 4838, 22.9%)
- rivaroxaban–ASA–Upper GI haemorrhage (n = 1040, 4.9%)
- rivaroxaban–clopidogrel–GI haemorrhage (n = 1009, 4.8%)

Of the 862 DDAs reviewed, 559 DDIs were not verified in the literature. A total of 41 PK DDIs and 265 PD DDIs were verified in the literature. The most common PK DDI was inhibition of drug metabolism, and the most common PD DDI was additive pharmacological effects.

Concerning verified PK DDIs, inhibitors of CYP3A and P-gp were the most reported drugs, and bleeding was the most reported ADR (Table 3). Regarding verified PD DDIs, antithrombotic agents and NSAIDs were the most reported drugs, and bleeding was also the most reported ADR. Regarding bleeding, the most reported site was the gastrointestinal tract (Table 3). Table 3 shows the number of occurrences that represent the number of different ADRs that occurred after the interaction between rivaroxaban and drug B, and the number in parentheses is the number of the most frequently reported ADR.

Table 3. Drug reported as interacting with rivaroxaban in VigiBase with interaction mechanism and most frequently reported adverse effect.

Drug B	No. of Occurrence	Mechanism	Mechanism Sub-Classification	Most Frequently Reported ADRs (No. Observed in Parenthesis)
Acetylsalicylic acid	48	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (4838)
Alendronic acid	1	PD	Additive pharmacological effect	Upper gastrointestinal haemorrhage (4)
Alteplase	2	PD	Additive pharmacological effect	Haemorrhagic stroke (4)
Amiodarone	8	PK	Drug metabolism (inhibition)	Haemorrhage (46)
Apixaban	5	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (102)
Azithromycin	2	PK	Drug metabolism (inhibition)	Pericardial haemorrhage (6)
Bosentan	1	PK	Drug metabolism (inhibition)	Anemia (3)
Carbamazepine	2	PK	Drug metabolism (induction)	Pulmonary embolism (6)
Celecoxib	8	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (56)
Ciprofloxacin	1	PK	Drug metabolism (inhibition)	Blood urine present (3)
Citalopram	1	PD	Additive pharmacological effect	Melaena (7)
Clarithromycin	1	PK	Drug metabolism (inhibition)	Haemorrhage subcutaneous (4)
Clopidogrel	25	PD	Additive pharmacological effect	Gastrointestinal haaemorrhage (1009)
Dabigatran	1	PD	Additive pharmacological effect	Internal haemorrhage (18)
Dalteparin	2	PD	Additive pharmacological effect	Haemorrhagic anemia (3) Muscle haemorrhage (3)
Diclofenac	8	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (40)
Dienogest/Ethinylestradiol	2	PD	Additive pharmacological effect	Menorrhagia (4)
Diltiazem	4	PK	Drug metabolism (inhibition)	Anemia (7)
Dipyrimadole	2	PD	Additive pharmacological effect	Cerebral haaemorrhage (3) Injection site haemorrhage (3)
Donepezil	2	PK	Drug metabolism (induction)	Cerebrovascular accident (3) Subdural haematoma (3)
Dronedarone	1	PK	Drug metabolism (inhibition)	Hematuria (6)
Drospirenone/ethinylestradiol	3	PD	Additive pharmacological effect	Deep vein thrombosis (6) Pulmonary embolism (6)
Duloxetine	1	PD	Additive pharmacological effect	Anemia (3)

Table 3. *Cont.*

Drug B	No. of Occurrence	Mechanism	Mechanism Sub-Classification	Most Frequently Reported ADRs (No. Observed in Parenthesis)
Eicosapetaenoic acid	1	PD	Additive pharmacological effect	Haemorrhage subcutaneous (3)
Enoxaparin	15	PD	Additive pharmacological effect	Rectal haemorrhage (57)
Escitalopram	4	PD	Additive pharmacological effect	Haematoma (5)
Etodolac	2	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (9)
Fluoxetine	2	PD	Additive pharmacological effect	Haematoma (4)
Fondaparinux	1	PD	Additive pharmacological effect	Haemorrhagic anemia (3)
Ginkgo biloba	3	PD	Additive pharmacological effect	Upper gastrointestinal haemorrhage (4)
Heparin	12	PD	Additive pharmacological effect	Rectal haaemorrhage (22)
Ibrutinib	3	PK/PD	Drug metabolism (inhibition) + additive pharmacological effect	Contusion (16)
Ibuprofen	16	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (161)
Iloprost	1	PD	Additive pharmacological effect	Haemorrhage (4)
Indometacin	5	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (12)
Itraconazole	2	PK	Drug metabolism (inhibition)	Ecchymosis (4) Epistaxis (4)
Ketoprofen	1	PD	Additive pharmacological effect	Anemia (9)
Ketorolac	2	PD	Additive pharmacological effect	Contusion (4)
Lenalidomide	1	PD	Additive pharmacological effect	Epistaxis (5)
Levonorgestrel	3	PD	Additive pharmacological effect	Menorrhagia (11)
Losartan	1	PK	Drug metabolism (inhibition)	Haemoglobin decreased (9)
Loxoprofen	1	PD	Additive pharmacological effect	Gastric ulcer haemorrhage (4)
Lubiprostone	1	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (3)
Meloxicam	6	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (70)
Metamizole	1	PD	Additive pharmacological effect	Upper gastrointestinal haemorrhage
Methylprednisolone	1	PD	Additive pharmacological effect	Anemia (3)
Nabumetone	1	PD	Additive pharmacological effect	Upper gastrointestinal haemorrhage (3)
Nadroparin	1	PD	Additive pharmacological effect	Hematuria (4)
Naproxen	11	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (135)
Paroxetine	3	PD	Additive pharmacological effect	Anemia (5)
Phenprocoumon	3	PD	Additive pharmacological effect	Hematochezia (4) Intestinal haemorrhage (4)
Pomalidomide	1	PK	Drug metabolism (inhibition)	Gastrointestinal haemorrhage (3)
Prasugrel	7	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (37)
Prednisolone	5	PD	Additive pharmacological effect	Anemia (5)
Prednisone	6	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (19)
Rifampicin	1	PK	Drug metabolism (induction)	Pulmonary embolism (8)
Riociguat	8	PD	Additive pharmacological effect	Epistaxis (30)
Sertraline	2	PD	Additive pharmacological effect	Anemia (4)
Sorafenib	1	PD	Additive pharmacological effect	Epistaxis (4)
Streptokinase	1	PD	Additive pharmacological effect	Haemorrhage (3)
Sunitinib	2	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (6)
Tadalafil	1	PK	Drug metabolism (inhibition)	Haemorrhage (4)
Ticagrelor	5	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (26)
Treprostinil	6	PD	Additive pharmacological effect	Haemorrhage (13)
Venlafaxine	2	PD	Additive pharmacological effect	Epistaxis (5)
Verapamil	2	PK	Drug metabolism (inhibition)	Haemoglobin decreased (3) Anemia (3)
Warfarin	21	PD	Additive pharmacological effect	Gastrointestinal haemorrhage (406)

4. Discussion

Due to their ease of use and alleged favorable safety and efficacy profile, anticoagulation drug management experienced a major turning point with the arrival of DOACs, especially rivaroxaban, which was the first to be marketed in 2009 for cardiovascular indications [14,91]. As rivaroxaban has been on the market for several years, it has been increasingly possible to highlight DDIs in real-world situations. In line with this, we performed a systematic review of published studies and case reports, together with an analysis of data reported to VigiBase, as already done with apixaban in a previous article [25]. We showed that rivaroxaban is subject to a significant number of DDIs that need to be considered by clinicians and patients, especially DDIs with CYP3A/P-gp inhibitors and other antithrombotic agents/NSAIDs. The impact of inducers of CYP3A/P-gp on rivaroxaban is sparsely available in the literature. A post hoc comparison between collected interactions in the literature and interactions contained in rivaroxaban's SmPC was performed to verify

the accuracy of our review [14]. First, the DDI between rivaroxaban and rifampicin reported in the rivaroxaban SmPC was not detected by our literature search and not registered in clinicaltrials.gov, which means that this study is not publicly available in any form and seems not to have even been registered in any national or international registry so far, even though registries of clinical trials are an important data source in clinical research. Conversely, some interactions that were identified by our search are not included in the SmPC. This can be explained by the fact that not all information has to be disclosed in the SmPC. Concerning in vitro interaction studies, data are only integrated into the SmPC if they impact the use of the medicinal product [61,92,93]. A lack of interaction should only be mentioned in the SmPC if it is of major significance to the prescriber for data from in vivo studies. Moreover, phase I studies in healthy volunteers publication depends on the transparency policies of drug manufacturers because they are not subject to required data disclosure [94,95]. Compared to studies performed in patients, a recent study showed that phase I (conducted in healthy volunteers) studies had a significantly lower level of transparency [95]. Finally, data from post-marketing studies are only included if they result in a variation of the drug's marketing authorisation [93,96].

Venous thromboembolism was identified in the case reports included in our literature search as one of the most frequently reported ADR of rivaroxaban, and it was not mentioned, per se, in rivaroxaban's SmPC. This is likely due to the fact that interactions leading to this ADR are not recognised and are instead classified as treatment inefficacy [20]. Therefore, this is not a lack of coverage in our literature search.

Regarding data from VigiBase, the most co-reported suspected/interacting drug was ASA, the most co-reported ADR was GI haemorrhage, and consequently, rivaroxaban–ASA–GI haemorrhage was the most reported DDA triplet. These results are not surprising, as multiple studies have highlighted the increased risk of GI haemorrhage when DOACs were administered, including a thorough evaluation of their safety profile based on data from the same source, VigiBase [19,20]. More precisely, rivaroxaban showed a positive odds ratio of 1.38 (1.24–1.55) for GI haemorrhage compared to warfarin [20]. Several suspected/interacting drugs were not documented or understood from a pharmacological point of view to be associated with a DDI with rivaroxaban, so they were excluded from our analysis of the ICSRs. Moreover, with the dataset available, it was not possible to find a plausible explanation for some of the DDIs, and many DDA triplets did not seem to correlate, such as rivaroxaban with mesalazine and poor-quality sleep. The data stored in VigiBase come from regulatory and voluntary sources and may lack a proper causality assessment in some cases, since not all national pharmacovigilance centres contributing to VigiBase perform causality assessments of their ICSRs [97]. Additionally, some cases may lack completeness, and the data stored are heterogeneous. Rivaroxaban might be at higher risk of interacting with drugs with the same pharmacological profile because the proportion of DDIs involving the PD mechanism was higher than the proportion of DDIs involving the PK mechanism. This finding erroneously suggests that rivaroxaban might not interact with CYP3A/P-gp inhibitors or inducers. Indeed, this emphasises a bias in the data included in VigiBase, which depends on spontaneous reporting. As healthcare professionals and/or patients are the source of these spontaneous reports and as they are often less familiar with PK DDIs, these are underreported because they go undetected. These results are consistent with those of a study that used the same database, where PD and PK DDIs accounted for 41% and 25% of DDIs, respectively [98].

VigiBase has inherent limitations, as all ADR reporting databases [99]. Underreporting and selective reporting are the two first limitations worth mentioning. Another limitation of these databases is the unfeasibility of estimating risk, due to the absence of a denominator. Using certain reporting patterns as indicators of DDIs in addition to a positive $\Omega_{0.25}$ is one of the ideas that have been put forward for improving the database [100]. The existence of a plausible time course, a positive dechallenge and alternate causes of the reaction could help identify suspected adverse drug interactions from ICSRs more precisely [101]. For that, it should be useful to take into account information available in the free text of the

original reports [101]. Nevertheless, the lack of completeness of each report is the root of the problem because not all fields are required to be completed for reports to be accepted in VigiBase, and a detailed case-by-case analysis of each ICSR is needed [102].

5. Conclusions

Contrary to what was mentioned at the time of marketing, rivaroxaban has significant DDI potential with other drugs. Data analysis of VigiBase and some articles in this review highlight that PD interactions, as well as drugs that may impair haemostasis such as ASA or antithrombotics, are widely known and reported. Indeed, they occur due to the known properties of the drug and are predictable. However, this literature review shows that rivaroxaban has particular DDI potential with CYP3A/P-gp inhibitors and CYP3A/P-gp inducers, but the analysis of VigiBase data shows that the detection and reporting of pharmacokinetic interactions are sparse because they are not well recognised. Moreover, SmPC does not contain all potentially described post-marketing DDIs. This should serve as a warning to healthcare professionals as to the likelihood of occurrence of ADRs due to DDIs, as they are avoidable.

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Chapter 3: Impact of the genotype and phenotype of CYP3A and P-gp on the apixaban and rivaroxaban exposure in a real-world setting.

Summary

Chapter 2 underlined that apixaban and rivaroxaban have significant potential for PK and PD DDIs. This chapter also highlighted that PK DDIs are under-detected and/or under-reported in real-world settings even though **chapter 1** explained that they are the most clinically significant DDIs. They encompass drugs that alter at least one step of the ADME process, but it is frequently acknowledged that the key contributor to the inter-individual variability in drug response is the alteration of drug metabolism.
This has been endorsed by **chapter 2** that observed that the main PK DDIs involved with apixaban and rivaroxaban occur with CYP3A and P-gp modulators. Indeed, as described in **chapter 1**, CYPs are the major DME and P-gp the most studied efflux transporter and their variability in activity and expression is explained by the influence of the genome and the exposome. The identification of the potential PK alterations should be known to personalize treatments and achieve an appropriate systemic exposure.

Based on these considerations, we assessed in the **research article 1** presented in **chapter 3** the impact of CYP3A and P-gp genetic polymorphisms and phenotypic activity on the blood concentrations of apixaban and rivaroxaban in a real-world population, i.e. hospitalized patients. The aim of the **chapter 3** is to clarify the use of CYP3A and P-gp genotype and phenotype testing during DOACs treatment to individualize treatment and optimize their benefit/risk balance. Detailed methods and results were published in the special issue « *Cardiovascular Disease Prevention in the Era of Personalized Medicine* » of the *Journal of Personalized Medicine*. Dramatic inter-individual variability was observed in dose-normalized blood concentrations and AUC of apixaban and rivaroxaban, as well as in CYP3A and P-gp activity metrics. P-gp phenotypic activity was significantly correlated to apixaban and rivaroxaban exposure and could therefore be considered as a relevant factor for apixaban and rivaroxaban treatments' dose adjustment, in addition to existing ones. However, CYP3A phenotype and CYP3A and P-gp SNPs tested had no significant impact on the PK of both molecules. The procedure of the genotyping test was explained in **chapter 1**.

My contributions to this **research article 1** were the entire management of the clinical study, the recruitment of patients, the collection of samples and data, the assessment of genotype, the analysis of the results and the article's writing.

Research article 1: Impact of the genotype and phenotype of CYP3A and P-gp on the apixaban and rivaroxaban exposure in a real-world setting.

Camille Lenoir, Jean Terrier, Yvonne Gloor, Pauline Gosselin, Youssef Daali, Christophe Combescure, Jules Alexandre Desmeules, Caroline Flora Samer, Jean-Luc Reny, Victoria Rollason.

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Article

Impact of the Genotype and Phenotype of CYP3A and P-gp on the Apixaban and Rivaroxaban Exposure in a Real-World Setting

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Abstract: Apixaban and rivaroxaban are the two most prescribed direct factor Xa inhibitors. With the increased use of DOACs in real-world settings, safety and efficacy concerns have emerged, particularly regarding their concomitant use with other drugs. Increasing evidence highlights drug–drug interactions with CYP3A/P-gp modulators leading to adverse events. However, current recommendations for dose adjustment do not consider CYP3A/P-gp genotype and phenotype. We aimed to determine their impact on apixaban and rivaroxaban blood exposure. Three-hundred hospitalized patients were included. CYP3A and P-gp phenotypic activities were assessed by the metabolic ratio of midazolam and $AUC_{0–6h}$ of fexofenadine, respectively. Relevant *CYP3A* and *ABCB1* genetic polymorphisms were also tested. Capillary blood samples collected at four time-points after apixaban or rivaroxaban administration allowed the calculation of pharmacokinetic parameters. According to the developed multivariable linear regression models, P-gp activity ($p < 0.001$) and creatinine clearance (CrCl) ($p = 0.01$) significantly affected apixaban $AUC_{0–6h}$. P-gp activity ($p < 0.001$) also significantly impacted rivaroxaban $AUC_{0–6h}$. The phenotypic switch (from normal to poor metabolizer) of P-gp led to an increase of apixaban and rivaroxaban $AUC_{0–6h}$ by 16% and 25%, respectively, equivalent to a decrease of 38 mL/min in CrCl according to the apixaban model. CYP3A phenotype and tested SNPs of CYP3A/P-gp had no significant impact. In conclusion, P-gp phenotypic activity, rather than known CYP3A/P-gp polymorphisms, could be relevant for dose adjustment.

Keywords: DOACs; pharmacogenomics; phenotype; metabolism; personalized medicine

1. Introduction

Apixaban and rivaroxaban are the two most prescribed direct oral anticoagulants (DOACs), both acting by direct inhibition of factor Xa (FXa) [1]. DOACs have become the treatment of choice for the treatment and prophylaxis of deep vein thrombosis (DVT) and pulmonary embolism (PE), as well as for the reduction of the risk of stroke and embolism in non-valvular atrial fibrillation (AF) [2–5]. Guidelines shifted from vitamin K antagonists (VKA) to DOACs, with DOACs being promoted as having a lower propensity to interact with drugs and food, a better predictable anticoagulant effect, and the ability

to be administered in fixed doses without routine monitoring [3,6]. Dose adjustment of DOACs is nonetheless required in specific risk groups [7]. For instance, dosing depends on indication, age (>80 years), body weight (<60 kg), and serum creatinine level (>1.5 mg/dL) for apixaban [2,8]. For rivaroxaban, dosing depends on indication and creatinine clearance (CrCl) values (CrCl < 50 mL/min) [2,9]. However, effectiveness and safety concerns in addition to significant inter-individual variations in dose–concentration response have been observed following their use in real-world settings, outside the strictly monitored conditions of the clinical trials [7,10].

Although apixaban and rivaroxaban are substrates of cytochrome P450 (CYP) 3A4/5 (3A) and P-glycoprotein (P-gp), variables impacting CYP3A and P-gp activity or expression (e.g., drug–drug interactions (DDIs) and genotypes) are not considered for dose adaptation [11]. This is a caveat, as the activity of drug transporters and metabolizing enzymes can be inhibited or induced by genetic, environmental, physiological, and pathophysiological factors, leading to DOACs' under- or overexposure [12]. The concern is important, as the risk of adverse drug reactions (ADRs) such as bleeding or thromboembolism increases with the occurrence of out-of-target concentrations [13]. It is also enhanced with polymedication, with a study showing that 30% of patients treated by DOACs received at least one interacting drug [7,14,15]. The increased bleeding risk due to coadministration with CYP3A and P-gp inhibitors is more and more reported in the literature, through case reports and several large registry-based retrospective studies [16–21]. The occurrence of thromboembolic events is also described in the literature after the concomitant use of apixaban and rivaroxaban with CYP3A and P-gp inducers [19,20]. Summaries of Product Characteristics (SmPC) only suggest avoiding concomitant use with strong CYP3A and P-gp inhibitors, without a benefit/risk evaluation [14,18]. There are currently no guidelines concerning coadministration with moderate or mild modulators, as data are rare and conflicting [14]. Numerous ADRs following DDIs involving apixaban or rivaroxaban and CYP3A/P-gp modulators were identified in VigiBase, the World Health Organization (WHO) database [19,20]. However, data on clinically relevant ADRs with DOACs due to DDIs and specific plasmatic concentrations inducing ADRs are scarce [22].

In addition to DDIs, the observed inter-individual variability in apixaban and rivaroxaban exposure could be related to polymorphisms of genes coding for CYP3A and/or P-gp, as recently reviewed [23]. Indeed, the effectiveness and safety of DOACs are influenced by genetically determined characteristics involved in drug metabolism [24]. For instance, a study found a significant association between the intronic variant *rs4148738* of *ABCB1* gene, coding for P-gp, and an increase in the peak concentration of apixaban [25]. Studies found that the presence of homozygous mutated TT genotype for *rs2032582* and *rs1045642* induced bleeding during rivaroxaban treatment [26]. However, other studies found that these variants had no significant impact [27]. The variants 1236C>T (*rs1128503*), 2677G>T (*rs2032582*), and 3435C>T (*rs1045642*) of the *ABCB1* gene had no impact on the concentration/dose ratio of apixaban [28]. Regarding CYP3A, the presence of CYP3A5*1/*3 or *3/*3 diplotypes was associated with an increase of apixaban concentration/dose ratio, compared to CYP3A5*1/*1 [28,29]. Nevertheless, conflicting results were reported, as a study found no significant impact of the CYP3A5*3 genetic polymorphism [30]. In addition, a study found that CYP3A4 activity had an impact on the peak and trough concentrations of rivaroxaban, while diverging results also exist [31,32].

Overall, selecting the suitable dose of DOACs is a complex process with different criteria and factors involved [33]. Data suggest a significant impact of factors altering CYP3A/P-gp activity, such as gene polymorphisms and DDIs, on rivaroxaban and apixaban exposure. In order to study the overall effect of such genetic and environmental effects on DOACs exposure, we used a validated cocktail approach with specific exogenous probes to prospectively determine whether CYP3A/P-gp phenotypic activities had a significant impact on apixaban and rivaroxaban exposure in hospitalized patients [7,13]. This real-life setting allowed us to ensure the establishment of a cohort displaying a large inter-individual variability in CYP3A/P-gp phenotypic activities caused by a broad panel of covariables.

This facilitated the study of their impact on DOAC exposure. The second aim of this study was to assess the impact of relevant gene polymorphisms for CYP3A/P-gp encoding genes on drug exposure.

2. Materials and Methods

2.1. Study Design

This study, investigating the impact of CYP3A and P-gp genotype and phenotype on blood concentrations of apixaban and rivaroxaban, was a real-life prospective observational study. The study protocol was registered on the US National Institutes of Health clinical trials registry (NCT03112525) and approved by the regional research ethics committee of the canton of Geneva (CCER) (No. 2016-01490, date of approval: 25 January 2017). Written informed consent was obtained from all participants prior to the initiation of any study procedure. The study complied with the principles of the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice Guidelines.

2.2. Study Population

Patients were recruited during their hospitalization at the Geneva University Hospitals between June 2017 and January 2021. Eligible patients were 18 years or above, diagnosed with AF, DVT, PE, and treated with apixaban or rivaroxaban for at least three days at the same dosage to ensure steady-state. Exclusion criteria included any known allergy to one of the components of the "Geneva cocktail" (caffeine, bupropion, flurbiprofen, omeprazole, dextromethorphan, midazolam, and fexofenadine). Patients were selected based on their electronic health record after a prescription alert was received for apixaban or rivaroxaban. Comedications were systematically screened to record patients taking CYP3A4/5 and/or P-gp inhibitors and/or inducers using the Lexicomp drug interaction analysis tool and the Geneva Table of CYP substrates, inhibitors, and inducers [34–36]. Adequacy of dosage was assessed according to the SmPC criteria (indication, age, CrCl, and weight).

2.3. Genotyping of CYP3A4/5 and P-Glycoprotein Encoding Genes

DNA was isolated from whole blood anticoagulated with EDTA, collected from each study participant prior to or following phenotype blood sampling, with a QIAsymphony® SP/AS (QIAGEN, Hilde, Germany) instrument using the QIAsymphony® DSP DNA Midi Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. Qubit™ fluorometer (ThermoFisher Scientific, Life Technologies Holdings Pte Ltd., Singapore) was used afterwards to quantify the purified DNA and ensure that the samples were at the normalized concentration of 30 ng/µL.

Genotyping of selected *CYP3A4/5* and *ABCB1* polymorphisms was carried out on QuantStudio™ 12K Flex Real-Time (RT) PCR System with TaqMan® OpenArray™ genotyping assays and TaqMan® MGB Probe Validated Single Nucleotide Polymorphism (SNP) Genotyping Assays (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA), respectively. These methods were previously described in detail in the literature [37–39]. In our study, SNPs rs1045642 (3435C>T), rs1128503 (1236C>T), and rs2032582 (2677G>T/A) of *ABCB1* were investigated. All SNPs of CYP3A4/5 integrated in the TaqMan® OpenArray™ PGx Express Panel (Thermo Fisher Scientific, Waltham, MA, USA) were considered and are listed in Supplementary Table S1. Finally, raw genotyping data were processed with the TaqMan® Genotyper software (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol.

AlleleTyper™ Software and translational tables (Thermo Fisher Scientific and PharmGKB, Stanford, CA, USA) were used to translate genetic pattern information from genotyping (SNPs) to pharmacogenomic gene-level star (*) nomenclature. Allele and genotype frequencies were determined, and Hardy–Weinberg equilibrium (HWE) was tested. The HWE is respected if the chi-squared (χ^2) value is less than 3.84, because 3.84 is the threshold value for a significance level of $p = 0.05$ for one degree of freedom. Samples with call rates below 95% were excluded from analysis. The CYP3A activity predicted from the

genotype combines the effects of CYP3A4 and CYP3A5 SNPs on enzyme activities, as listed in the PharmVar and PharmGKB databases [40,41]. Patients were classified into poor metabolizer (PM), intermediate metabolizer (IM), normal metabolizer (NM), and ultra-rapid metabolizer (UM) according to the classification described by Andreu et al. [42].

2.4. Phenotyping

The phenotypic activity of CYP3A4/5 and P-gp was measured by calculating the metabolic ratio (MR) and the area under the curve ($AUC_{0–6h}$) of the probe substrates, respectively. The "Geneva cocktail" (caffeine 50 mg, CYP1A2; bupropion 20 mg, CYP2B6; flurbiprofen 10 mg, CYP2C9; omeprazole 10 mg, CYP2C19; dextromethorphan 10 mg, CYP2D6; midazolam 1 mg, CYP3A; and fexofenadine 25 mg, P-gp) was administered orally on an empty stomach. Capillary blood samples were collected two (t + 2 h), three (t + 3 h), and six (t + 6 h) hours later with dried blood spots (DBS), using a previously validated sampling method, and were stored at −20 °C in a sealable plastic bag until analysis [43,44]. MR of CYP3A4/5 consists of the blood concentration of 1-OH-midazolam divided by the blood concentration of midazolam measured after two hours. The activity of P-gp is derived from the $AUC_{0–6h}$ of fexofenadine ($AUC_{fexofenadine}$), calculated by linear trapezoidal rule using WinNonlin® version 6.2.1 (Pharsight, Mountain View, CA, USA) from blood concentrations of fexofenadine measured at t + 2 h, t + 3 h, and t + 6 h. A previously validated method using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) quantification was used to assess the blood concentrations of midazolam, 1-OH-midazolam, and fexofenadine [43,45,46].

2.5. Laboratory Markers Levels

Whole-blood samples with lithium heparin were collected early in the morning on the study day to assess liver and renal function. The concentration of aspartate transaminase (ASAT), alanine transaminase (ALAT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), bilirubin, and creatinine were measured directly after blood sampling. The CrCl was calculated according to the Cockcroft–Gault formula and used as a continuous variable in our multivariable linear regression models. However, to describe the population, patients were classified based on their CrCl into normal (>60 mL/min/1.73 m^2), moderate (30 < x < 59 mL/min/1.73 m^2), severe (15 < x < 29 mL/min/1.73 m^2), and end-stage renal disease (<15 mL/min/1.73 m^2). Patients were also classified into normal or abnormal liver function (defined as ASAT, ALAT, bilirubin, GGT > 2 × upper limit of normal).

2.6. Apixaban and Rivaroxaban Blood Concentrations

Capillary blood samples (10 µL) were collected in DBS just before apixaban and rivaroxaban administration (t0) and at t + 2 h, t + 3 h, and t + 6 h. The DBS concentrations were determined using a validated LC-MS/MS method. The instrumentation used was composed of an Agilent 1290 Infinity series LC system from Agilent (Paolo Alto, Santa Clara, CA, USA) coupled to a 6500 QTrap® triple quadrupole linear ion trap mass spectrometer from AB Sciex equipped with an electrospray ionization (Darmstadt, Germany).

Before analysis, discs (i.d. 8 mm) covering the entire DBS were punched out, placed in LC vials, and extracted by adding 100 µL of methanol containing 200 ng/mL of internal standards (apixaban-d3 and rivaroxaban-d4). After agitation during 10 min, 10 µL of the supernatant was injected into the LC-MS/MS system. Separation was performed with a Kinetex® C18 column (50 × 2.1 mm, 2.6 µm) from Phenomenex (Brechbühler, Schlieren, Switzerland) under gradient conditions. The mobile phase was composed of formic acid 0.1% in water and in acetonitrile. The total run time was 7 min. Detection of analytes was obtained in positive mode using multiple reaction monitoring (MRM). Instrument parameters were as follows: curtain gas = 40 psi, collision gas = high, IonSpray voltage = 4500 kV, temperature = 550 °C, ion source gas 1 = 60 psi, ion source gas 2 = 60 psi. The transitions monitored for each analyte (precursor ion > product-fragment ions) were: apixaban

460.1 > 443.1, apixaban-d3 463.1 > 446.1, rivaroxaban 436.0 > 144.8, and rivaroxaban-d4 440.1 > 144.8. The optimized collision energy was +33 V for apixaban and +35 V for rivaroxaban. Declustering potential (DP) was +156 V for apixaban and +136 V for rivaroxaban. Cell exit potential (CXP) was +36 V for apixaban and +16 V for rivaroxaban.

The calibration curves were linear over the standard concentration ranges of 1–1000 ng/mL for all analytes and trueness; inter and intraday variabilities were in line with the validation guidelines of the European Medicines Agency. The $AUC_{0–6h}$ of apixaban and rivaroxaban were calculated by linear trapezoidal rule using WinNonlin® version 6.2.1 (Pharsight, Mountain View, CA, USA).

2.7. Statistical Analysis

A sample size of 204 patients treated with apixaban was needed to detect a difference of at least 50 ng/mL in mean plasma concentration of apixaban between patients with high or low enzymatic activity (CYP3A4/5 and P-gp) with a power of 80% and a two-sided α-value of 5%. A standard deviation (SD) of 100 ng/mL in each group was assumed.

Concerning rivaroxaban, a sample size of 150 patients treated with rivaroxaban was needed to detect a difference of at least 60 ng/mL in mean plasma concentration of rivaroxaban between patients with high or low enzymatic activity (CYP3A4/5 and P-gp) with a power of 80% and a two-sided α-value of 5%. A SD of 100 ng/mL in each group was assumed. For both molecules, a normal CYP3A4/5 activity, as predicted by the genotype, was expected in 20% of enrolled patients. Indeed, the CYP3A5*3 mutation has a reduced activity and is highly prevalent in the population worldwide, especially in Caucasians [47].

Dependent variables (outcomes) for both drugs were defined as being the values of $AUC_{0–6h}$ and concentration 2 h after drug administration (C_{2h}). All statistical analyses were performed using the software R version 4.0.2 (R Core Team, 2016. R: A Language and Environment for Statistical Computing, Vienna, Austria), and a p-value < 0.05 was considered statistically significant. Means ± SD were used to describe continuous variables. Associations of variables with outcomes were investigated with multivariable linear regression models. Each independent variable is reported with its beta coefficient (β) and its 95% confidence interval (CI95%). For continuous independent variables ($MR_{midazolam}$, $AUC_{fexofenadine}$, BMI, CrCl, and age), the linearity of the relationship was graphically inspected. Because of the skewness of the distribution of $MR_{midazolam}$ and $AUC_{fexofenadine}$, a $\log_{10}$ transformation was applied. The Breusch–Pagan test was used to detect a potential heteroscedasticity issue, and consistent standard errors of the regression coefficients were assessed with a sandwich estimator. Association between dependent variables and phenotypic activity of CYP3A and P-gp ($MR_{midazolam}$ and $AUC_{fexofenadine}$, respectively) were adjusted for a pre-specified set of potential confounding factors (gender, CrCl, BMI, age, dose). In addition, association between dependent variables and predicted activity of CYP3A and P-gp from genotype were also adjusted for a pre-specified set of potential confounding factors (gender, CrCl, BMI, age, dose). Spearman's correlation was used to assess the concordance between genotype and phenotype of CYP3A and P-gp. Missing data were excluded from the analysis.

3. Results

3.1. Demographics

Overall, 300 patients were included, with 164 receiving apixaban and 136 receiving rivaroxaban. Although lower than anticipated, sample sizes allowed detection of a difference of at least 57 ng/mL and 62 ng/mL in mean plasma concentration of apixaban and rivaroxaban, respectively, with a power of 80% and a two-sided α-value of 5%. The only patient receiving rivaroxaban 2.5 mg twice daily (bid), a new indication in association with aspirin for the prevention of atherothrombotic events in high-risk patients, was removed from the analysis. Demographic characteristics of the study population are presented in Table 1.

Table 1. Demographic characteristics of included patients with apixaban and rivaroxaban treatments.

Characteristics	Apixaban	Rivaroxaban
Gender, *n* (%)		
Male	101 (61.6%)	89 (65.9%)
Female	63 (38.4%)	46 (34.1%)
Age, mean (SD)		
Age (years)	77.4 (9.8)	71.1 (12.1)
Weight, mean (SD)		
Weight (kg)	77.4 (9.8)	82.1 (18.0)
BMI (kg/m^2)	26.8 (5.6)	27.7 (5.7)
Indication, *n* (%)		
AF	145 (88.4%)	92 (67.6%)
DVT and PE treatment	13 (7.9%)	35 (25.7%)
DVT and PE prophylaxis	6 (3.7%)	9 (6.6%)
Doses, *n* (%)		
2.5 mg bid	70 (42.7%)	NA
5 mg bid	87 (53%)	NA
10 mg bid	7 (4.3%)	NA
10 mg od	NA	6 (4.4%)
15 mg od	NA	17 (12.6%)
20 mg od	NA	80 (59.3%)
15 mg bid	NA	32 (23.7%)
Dosage adequacy, *n* (%)		
Adequate dosage	166 (70.7%)	108 (80%)
Inadequate dosage	47 (28.7%)	27 (20%)
Unknown	1	0
Inadequate dosage, *n* (%)		
2.5 mg bid	45 (95.7%)	NA
10 mg od	NA	3 (11.1%)
15 mg od	NA	9 (33.3%)
20 mg od	NA	9 (33.3%)
15 mg bid	NA	6 (22.2%)
AF	43 (91.5%)	22 (81.5%)
Liver injury, *n* (%) or mean (SD)		
ALAT	34.5 (46.7)	38.9 (41.6)
No	149 (92.5%)	122 (90.4%)
Yes	12 (7.5%)	13 (9.6%)
Missing data	3	0
Renal function, *n* (%) or mean (SD)		
Creatinine (µ/moL)	110.8 (111.4)	95.8 (88.3)
CrCl (mL/min/1.73 m^2) according to Cockcroft	63.8 (27.5)	77.7 (28.0)
Normal	75 (46.0%)	91 (67.4%)
Moderate	79 (48.5%)	43 (31.9%)
Severe	9 (5.5%)	1 (0.7%)
Missing data	1	0

Abbreviations: SD, standard deviation; BMI, body mass index; AF, atrial fibrillation; DVT, deep vein thrombosis; PE, pulmonary embolism; bid, twice daily; od, once daily; NA, not applicable; ALAT, alanine transaminase; CrCl, creatinine clearance.

3.2. Genotypes

Of the 299 remaining patients, 294 patients were successfully genotyped. Frequencies for each SNP studied are presented in Table 2. Five patients were not genotyped due to

missing blood sampling. Predicting P-gp phenotype from genotype was impossible because of the lack of clear haplotype–phenotype correlations. The final sample and SNP call rates for the whole analysis (CYP3A4, CYP3A5, and ABCB1) was 99.6% and 99.4%, respectively. No significant departure from HWE was found for all the SNPs, except for CYP3A4*1B ($\chi^2 = 11.25$, $p = 0.001$). The frequencies of the different genotypes were relatively similar between the two drugs studied and correspond to the reference population (Caucasian) [47]. Data are shown in Supplementary Table S2.

Table 2. Genotype analysis results.

Characteristics	Apixaban	Rivaroxaban
Predicted phenotype from genotype CYP3A, *n* (%)		
PM	18 (11.3%)	13 (10.1%)
IM	127 (79.9%)	99 (76.7%)
NM	14 (8.8%)	17 (13.2%)
Missing data	5	6
Genotype *ABCB1* 1236C>T, *n* (%)		
No T	52 (32.1%)	44 (33.6%)
One T	74 (45.7%)	62 (47.3%)
Two T	36 (22.2%)	25 (19.1%)
Missing data	2	4
Genotype *ABCB1* 2677G>T, *n* (%)		
No T	49 (30.4%)	43 (33.1%)
One T	75 (46.6%)	56 (43.1%)
Two T	37 (23.0%)	31 (23.8%)
Missing data	3	5
Genotype *ABCB1* 3435C>T, *n* (%)		
No T	41 (25.5%)	36 (27.5%)
One T	74 (46.0%)	58 (44.3%)
Two T	46 (28.6%)	37 (28.2%)
Missing data	3	4

Abbreviations: PM, poor metabolizer; IM, intermediate metabolizer; NM, normal metabolizer; T, mutant allele.

3.3. Phenotypes

All the included patients received the Geneva cocktail, but the fexofenadine $AUC_{0–6h}$ calculation is missing for one patient who received rivaroxaban treatment, because the sampling at t + 3 h and t + 6 h was not possible. The means ± SD of $MR_{midazolam}$ in the apixaban and rivaroxaban cohorts are 0.62 ± 0.67 and 0.58 ± 0.58, respectively. The means ± SD of $AUC_{fexofenadine}$ in the apixaban and rivaroxaban cohorts are 265.0 ± 178.0 ng × h/mL and 237.9 ± 170.0 ng × h/mL, respectively. Results are summarized in Figure 1; Figure 2 for apixaban and rivaroxaban, respectively. The calculated inter-individual variability (coefficient of variation (CV)) of $MR_{midazolam}$ and $AUC_{fexofenadine}$ for the apixaban cohort is of 108.1% and 67.2%, respectively. The CV of $MR_{midazolam}$ and $AUC_{fexofenadine}$ for the rivaroxaban cohort is of 100.0% and 71.5%, respectively. Spearman's correlation coefficient between $MR_{midazolam}$ and $AUC_{fexofenadine}$ is $\rho = -0.271$ ($p < 0.0001$).

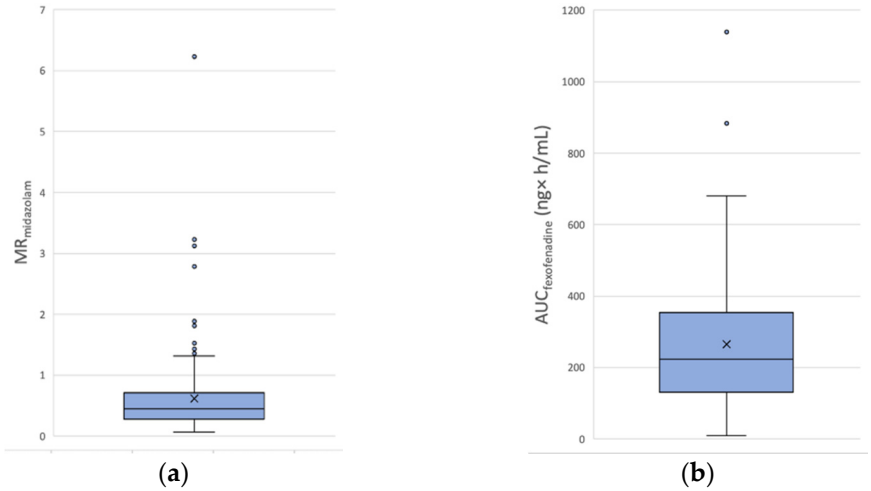


Figure 1. Distribution of phenotype metrics in the apixaban cohort (**a**) $MR_{midazolam}$ and (**b**) $AUC_{fexofenadine}$.

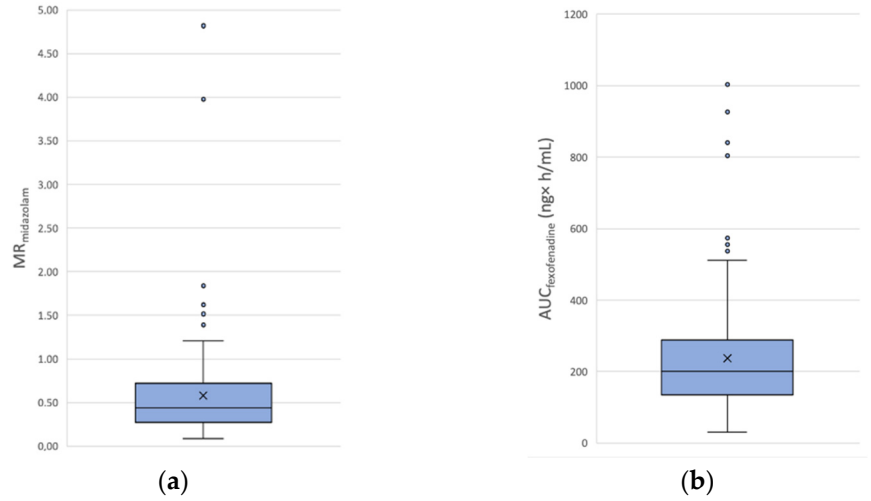
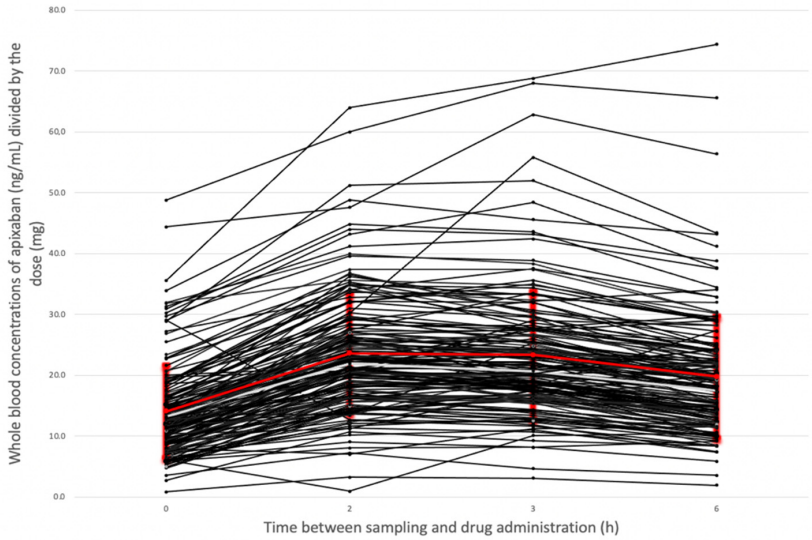


Figure 2. Distribution of phenotype metrics in the rivaroxaban cohort (**a**) $MR_{midazolam}$ and (**b**) $AUC_{fexofenadine}$.

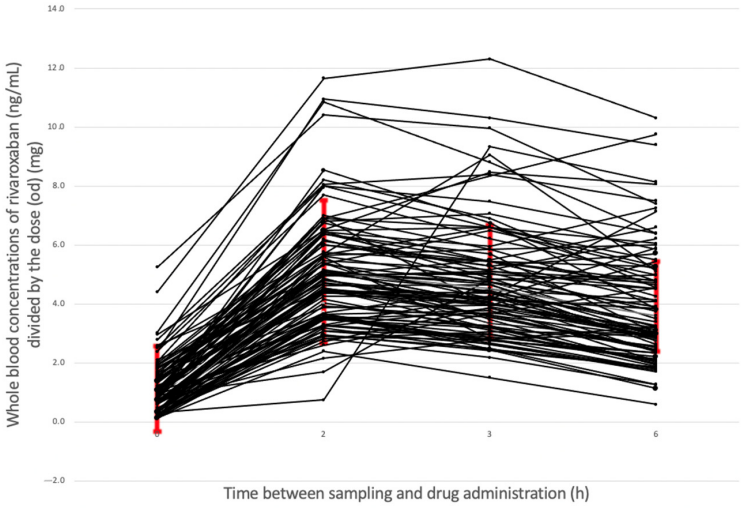
3.4. Apixaban and Rivaroxaban Blood Concentrations

Individual pharmacokinetic (PK) profiles for patients treated with apixaban bid, rivaroxaban once daily (od), and rivaroxaban bid are presented in Figure 3a–c, respectively. The corresponding mean ± SD is highlighted in red. All blood concentrations were normalized by the dosing regimen. The inter-individual CV of blood concentrations is 47.7% for apixaban bid, 51.8% for rivaroxaban administered od, and 41.5% for rivaroxaban administered bid.

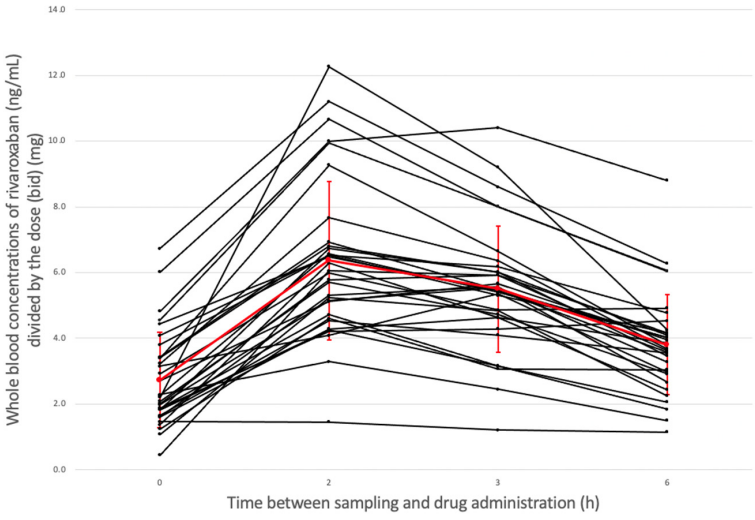
Spearman's correlation coefficient between blood concentrations at t + 2 h and t + 3 h were $\rho = 0.94$ ($p < 0.0001$) and $\rho = 0.82$ ($p < 0.0001$) for apixaban and rivaroxaban, respectively. As we observed a good correlation, the C_{2h} was used for the analysis.



(**a**)



(**b**)



(**c**)

Figure 3. Blood concentrations normalized by the dosing regimen of (**a**) apixaban (**b**) rivaroxaban once daily and (**c**) and rivaroxaban twice daily. Each black line corresponds to an individual, and the mean ± SD is highlighted in red.

3.5. Multivariable Linear Regression

A multivariable linear regression model was built to assess the factors associated with the $AUC_{0–6h}$ or C_{2h} of apixaban and rivaroxaban. The models built for the $AUC_{0–6h}$ of apixaban and rivaroxaban are shown in Table 3 and predict 47% and 27% of the observed variability, respectively.

Table 3. Multivariable linear regression models to assess if the phenotypic activity of CYP3A and P-gp are associated with the $AUC_{0–6h}$ of apixaban and rivaroxaban. Each independent variable is reported with its beta coefficient (β) and its 95% confidence interval (CI95%).

	$AUC_{0–6h}$ of Apixaban	**$AUC_{0–6h}$ of Rivaroxaban**
Intercept	−46.30 (−339.86 to 247.26); $p = 0.7557$	−418.12 (−776.92 to −59.32); $p = 0.0228$
Variables		
$MR_{midazolam}$, per log_{10}	10.03 (−64.67 to 84.72); $p = 0.7912$	−90.27 (−209.99 to 29.45); $p = 0.1381$
$AUC_{fexofenadine}$, per log_{10}	173.96 (77.33 to 270.58); **$p = 0.0005$**	232.51 (105.69 to 359.33); **$p = 0.0004$**
Weight, per kg	−0.25 (−2.12 to 1.61); $p = 0.7881$	1.19 (−0.63 to 3.01); $p = 0.1973$
CrCl, per unit	−2.13 (−3.72 to −0.54); **$p = 0.0091$**	−0.44 (−2.53 to 1.65); $p = 0.6675$
ALAT, per unit	0.44 (−0.91 to 1.79); $p = 0.5217$	0.08 (−0.65 to 0.81); $p = 0.8314$
Gender		
Male	Reference category	Reference category
Female	43.31 (−15.31 to 101.92); $p = 0.1464$	44.63 (−27.63 to 116.89); $p = 0.2238$
Dose		
2.5 mg bid	Reference category; **$p < 0.0001$ ***	NA
5 mg bid	279.44 (221.14 to 337.75); $p < 0.0001$	NA
10 mg bid	688.81 (478.01 to 889.60); $p < 0.0001$	NA
10 mg od	NA	Reference category; **$p = 0.0045$ ***
15 mg od	NA	90.97 (−32.04 to 213.99); $p = 0.1457$
20 mg od	NA	165.18 (37.54 to 292.83); $p = 0.0116$
15 mg bid	NA	180.44 (64.84 to 296.02); $p = 0.0025$
Age		
<65 years	Reference category; $p = 0.2064$ *	Reference category; $p = 0.1191$ *
65–74 years	95.82 (−10.13 to 201.76); $p = 0.0759$	81.34 (15.35 to 147.32); $p = 0.0161$
75–84 years	82.33 (−22.35 to 187.01); $p = 0.1222$	59.56 (−53.36 to 172.48); $p = 0.2984$
>85 years	113.34 (−1.13 to 227.82); $p = 0.0523$	56.06 (−54.25 to 166.38); $p = 0.3163$

* p-value for the overall association between $AUC_{0–6h}$ and the variable. Abbreviations: CrCl, creatinine clearance; ALAT, alanine transaminase; AUC, area under the curve; bid, twice daily; od, once daily; NA, not applicable. Statistically significant values are marked with bold.

The models built for the C_{2h} of apixaban and rivaroxaban are shown in Supplementary Table S3.

After adjustment for all the covariables, the P-gp activity and the dose administered have a positive and significant association with $AUC_{0–6h}$ and C_{2h} of apixaban and rivaroxaban. In addition, the CrCl is negatively and significantly associated to $AUC_{0–6h}$ and C_{2h} of apixaban, while this is not the case with rivaroxaban. In practice, an increase in fexofenadine $AUC_{fexofenadine}$ from 100.1 ng × h/mL to 285.5 ng × h/mL (corresponding to a phenotype conversion from NM to PM according to our inner threshold values) would lead to an increase in apixaban and rivaroxaban $AUC_{0–6h}$ by about 16% and 25%, respectively [43,44]. For apixaban, this P-gp phenoconversion can be compared to the effect of a decrease in CrCl of 37.6 mL/min/1.73 m^2. For rivaroxaban, this phenoconversion is equivalent to an increase in the dose category (see Table 3).

Age, gender, weight, ALAT level, and CYP3A activity were not associated with $AUC_{0–6h}$ and C_{2h} variations of apixaban and rivaroxaban.

The same multivariable linear regression models were built to assess the impact of activity predicted from genotype of CYP3A and P-gp with the PK parameters of apixaban and rivaroxaban. Models built for the $AUC_{0–6h}$ of apixaban and rivaroxaban are shown in Table 4 and explain approximately 40% and 18% of the observed variability, respectively.

Table 4. Multivariable linear regression models to assess whether the genotype of CYP3A and P-gp are associated with the $AUC_{0–6h}$ of apixaban and rivaroxaban. Each independent variable is reported with its beta coefficient (β) and its 95% confidence interval (CI95%).

	$AUC_{0–6h}$ of Apixaban	**$AUC_{0–6h}$ of Rivaroxaban**
Intercept	398.75 (196.35 to 601.15); p = 0.0002	−39.45 (−424.81 to 345.91); p = 0.8396
Variables		
Weight, per kg	−0.11 (−2.08 to 1.86); p = 0.9142	1.89 (0.05 to 3.72); ***p* = 0.0442**
CrCl, per unit	2.38 (−3.97 to −0.79); ***p* = 0.0036**	−0.43 (−2.90 to 2.05); p = 0.7333
ALAT, per unit	0.45 (−1.20 to 2.11); p = 0.5893	0.34 (−0.35 to 1.04); p = 0.3280
Predicted phenotype from genotype CYP3A		
IM	Reference category; p = 0.2154 *	Reference category; ***p* = 0.0021 ***
NM	−15.50 (−139.46 to 108.47); p = 0.8051	142.49 (56.08 to 228.90); p = 0.0014
PM	−77.10 (-164.78 to 10.58); p = 0.0843	134.23 (−24.82 to 293.28); p = 0.0973
Genotype *ABCB1* 1236C>T		
No mutation	Reference category; p = 0.9723 *	Reference category; p = 0.4955 *
Heterozygous for mutation	−12.33 (−138.96 to 114.30); p = 0.8476	−46.50 (−163.59 to 70.59); p = 0.4329
Homozygous for mutation	−20.09 (−190.52 to 150.34); p = 0.8160	21.46 (−125.94 to 168.86); p = 0.7735
Genotype *ABCB1* 3435C>T		
No mutation	Reference category; p = 0.5600 *	Reference category; p = 0.2663 *
Heterozygous for mutation	−51.58 (−149.70 to 46.54); p = 0.3004	−51.69 (−170.92 to 67.54); p = 0.3921
Homozygous for mutation	−18.22 (−112.45 to 76.01); p = 0.7028	−71.90 (−161.27 to 17.46); p = 0.1137

Table 4. *Cont.*

	$AUC_{0–6h}$ of Apixaban	$AUC_{0–6h}$ of Rivaroxaban
Genotype *ABCB1* 2677G>T		
No mutation	Reference category; $p = 0.9069$ *	Reference category; $p = 0.6892$ *
Heterozygous for mutation	29.83 (−106.91 to 166.57); $p = 0.6669$	56.52 (−75.24 to 188.29); $p = 0.3971$
Homozygous for mutation	32.15 (−138.11 to 202.40); $p = 0.7095$	54.86 (−96.09 to 205.81); $p = 0.4728$
Gender		
Male	Reference category	Reference category
Female	50.24 (−19.91 to 120.38); $p = 0.3004$	46.71 (−29.08 to 122.49); $p = 0.2246$
Dose		
2.5 mg bid	Reference category; **$p < 0.0001$ ***	NA
5 mg bid	275.77 (201.67 to 349.87); $p < 0.0001$	NA
10 mg bid	689.14 (470.72 to 907.55); $p < 0.0001$	NA
10 mg od	NA	Reference category; **$p = 0.0012$ ***
15 mg od	NA	128.66 (−17.23 to 274.55); $p = 0.0833$
20 mg od	NA	250.76 (117.30 to 384.22); $p = 0.0003$
15 mg bid	NA	221.57 (75.08 to 368.07); $p = 0.0034$
Age		
<65 years	Reference category; $p = 0.3989$ *	Reference category; **$p = 0.0062$ ***
65–74 years	81.29 (−37.74 to 200.33); $p = 0.1719$	138.27 (61.87 to 214.67); $p = 0.0005$
75–84 years	57.29 (−50.00 to 164.58); $p = 0.2929$	127.49 (−17.50 to 272.48); $p = 0.0842$
>85 years	104.66 (−32.37 to 241.70); $p = 0.1333$	124.29 (−14.68 to 263.25); $p = 0.0791$

* p-value for the overall association between $AUC_{0–6h}$ and the variable. Abbreviations: CrCl, creatinine clearance; ALAT, alanine transaminase; IM, intermediate metabolizer; NM, normal metabolizer; PM, poor metabolizer; bid, twice daily; od, once daily; NA, not applicable. Statistically significant values are marked with bold.

The models built for the C_{2h} of apixaban and rivaroxaban are shown in Supplementary Table S4.

No SNP of P-gp tested was found to be associated with apixaban and rivaroxaban AUC_{0-6h} (Table 4) or C_{2h} (Supplementary Table S4). The CYP3A predicted activity from genotype was found to be associated with AUC_{0-6h} (Table 4) or C_{2h} (Supplementary Table S4) of rivaroxaban but not apixaban. However, these results showed that being NM paradoxically increases the exposure to rivaroxaban, as compared to IM (Supplementary Figure S1). It highlights the difficulty to predict the CYP3A phenotype from genotype, which is confirmed by the absence of correlation between CYP3A predicted by genotype and $MR_{midazolam}$, as shown in Supplementary Table S5. This table also shows that SNPs of P-gp was not associated with $AUC_{fexofenadine}$. This means that there is no concordance between genotype and phenotype in our study for CYP3A and P-gp.

Similar to previous models for phenotype activity predicted by the cocktail approach, CrCl and dose were found to be associated with apixaban PK parameters and only with dose

for rivaroxaban. However, age and weight were also found to be significantly associated with rivaroxaban $AUC_{0\text{-}6h}$ and C_{2h}.

4. Discussion

Our study found that P-gp phenotypic activity impacts apixaban and rivaroxaban exposure. To the best of our knowledge, it is the first time that a metric of P-gp phenotypic activity has been linked to apixaban and rivaroxaban concentrations in vivo. These results support the important role played by P-gp in the PK process of these two drugs in vivo [11,18]. P-gp is an efflux transporter that pumps the absorbed drugs from inside the enterocyte back into the intestinal lumen, decreasing the net gut absorption [48,49]. Despite the ability of P-gp to cause the elimination of apixaban and rivaroxaban into the gut lumen, recently published in silico studies suggested that the intestinal P-gp is not clinically significant in the absorption process of apixaban and rivaroxaban [49,50]. However, these studies have some limitations, such as using mean concentration–time curves rather than individual PK profiles and PK curves with possibly insufficient time points during the absorption phase [49,50]. Moreover, a recent in vitro study used human renal cells to provide data on P-gp inhibition and showed that P-gp had a main role in the efflux of apixaban and rivaroxaban [51].

Inter-individual variability in P-gp phenotype activity can result from the influence of both exposome and/or genome [52–56]. Many environmental factors influence P-gp activity in hospitalized populations like ours, especially DDIs and disease state. Our results thus question whether dose adaptation should be suggested for apixaban and rivaroxaban in the presence of P-gp modulators. Currently, dose adjustment is not required when a P-gp modulator is co-administered with apixaban and rivaroxaban, but it is with edoxaban [57,58]. Indeed, edoxaban was the only DOAC for which such dose adaptation was validated in its major trial (ENGAGE AF-TIMI 48), unlike the major trials on apixaban and rivaroxaban (ARISTOTLE AF and ROCKET AF trials, respectively), which excluded potent P-gp inhibitors [59–61].

The present study suggests that a phenotypic switch of P-gp activity from NM to PM might result in an increase in apixaban and rivaroxaban $AUC_{0–6h}$ by 16% and 25%, respectively [43,44]. This is far from the two- to five-fold increase in the AUC of a substrate with a specific inhibitor to allow it being classified as even a moderate inhibition [62]. However, this result is clinically relevant, as its magnitude is equivalent to a decrease in the renal function category in apixaban and an increase in the dosing regimen for rivaroxaban, according to our models. Overall, our results suggest that dose adaptation should be questioned in the presence of P-gp modulators.

Other important environmental factors impacting CYP3A/P-gp phenotype activity are the inflammation state and the renal insufficiency, particularly in hospitalized patients. Growing evidence suggests that these alter CYP/P-gp activity through cytokines and uremic toxins, respectively [53,54,63–67]. This may have been a confounding factor that led to the loss of significance of the effect of modulation of CYP3A on apixaban and rivaroxaban $AUC_{0–6h}$ in our model. The effect of inflammation and renal insufficiency on P-gp activity has received less attention, but some evidence suggests an alteration of the P-gp depending on intensity, time, and isoform considered [53,68–70].

The absence of association between CYP3A phenotypic activity and the apixaban and rivaroxaban exposure could result from different and complementary scenarios. As CYP3A is responsible for only 15% and 18% of the metabolism of apixaban and rivaroxaban, respectively, modulation of this pathway could, therefore, have a small impact [11]. Moreover, many CYP3A modulators are also P-gp modulators, and pure CYP3A4/5 modulation may only have a modest effect on apixaban and rivaroxaban metabolism [52]. This highlights the need to further investigate the clinical relevance of combined versus single CYP3A4/P-gp modifier interactions, as studies that distinguish the relative contribution of P-gp as compared to CYP3A modulation for each known DDI are lacking [22,52,71]. This is exemplified by the fact that FDA guidelines propose to assess drug transporters

modulation only if this drug clinically modulates CYP3A [71]. Consequently, PK studies and SmPCs of apixaban and rivaroxaban mention almost every time the combined effect of P-gp and CYP3A modulators and not each enzyme separately [11,14,18–20]. For instance, the drugs established to be responsible for thrombotic and bleeding events are mostly classified as CYP3A4/P-gp inducers and inhibitors, respectively [22,72]. However, P-gp induction is limited to human in vitro data, resulting in the assumption that the DDIs are solely attributed to a CYP3A induction [22]. Authors of a recent systematic review suggest that it is the combination of CYP3A4/P-gp inhibitors that lead to bleeding events, while a single CYP3A4 inducer or the combination of CYP3A4/P-gp inducers were responsible for thrombotic events [22]. This is in line with our results, where the impact of P-gp inhibition was more potent in increasing apixaban and rivaroxaban blood concentrations than inhibition of CYP3A alone. In addition, we observed almost no induction of CYP3A activity in our study, and this may have weakened the significance of the impact of CYP3A alone. However, the absence of association between the CYP3A phenotype activity and the PK of apixaban and rivaroxaban does not come from the absence of sufficient inter-individual variability in CYP3A phenotype activity. Indeed, the calculated coefficients of variation of CYP3A activity are 108.1% and 100.0% for the apixaban and rivaroxaban cohorts, respectively, which ensures a high inter-individual variability. The calculated coefficients of variation of P-gp activity are smaller, with 67.2% for the apixaban and 71.5% for the rivaroxaban cohorts. It is indeed known that CYP3A carries a five-fold constitutive variability due to its sensitivity to multiple factors [73]. Moreover, not all CYP3A substrates share the same specificity, and it cannot be excluded that midazolam is not a good surrogate for DOACs [74].

Overall, PK profiles appear to be significantly impacted when multi-target inhibitors are administered. Apixaban and rivaroxaban are also substrates of Breast Cancer Resistance Protein (BCRP), encoded by the *ABCG2* gene, which is another efflux transporter [23]. Two studies observed that the SNPs rs2231142 of *ABCG2* (c.421C > A) had an impact on apixaban exposure, and one case report showed a highly increased concentration of apixaban, along with other mutations on *ABCB1* and *CYP3A5* gene [28,75,76]. However, this ABCG2 efflux transporter is present in the intestine and does not seem to have a significant impact on absorption of apixaban [77]. Concerning rivaroxaban, the c.421C > A *ABCG2* mutation does not seems to have any impact, while some potential BCRP inhibitors showed an interaction with rivaroxaban [78,79]. Nevertheless, these potential BCRP inhibitors are also CYP3A and/or P-gp inhibitors. In the future, it could be interesting to measure the BCRP expression or to assess its genotype.

Another CYP is involved in the metabolism of apixaban and rivaroxaban, named CYP2J2 [23,78]. It contributes to 14% of the total clearance of rivaroxaban, which is approximately the same as the CYP3A [11]. The catalytic efficiency of CYP2J2 was assessed to be higher than that of CYP3A4 in vitro, giving a new insight of DDIs involving rivaroxaban [80]. CYP2J2*7 did not significantly impact the exposure to rivaroxaban, as observed in a study [78]. Other genetic polymorphisms of CYP2J2 have been identified, but their clinical implications are to date unknown [80,81]. For instance, ketoconazole and ritonavir have been reported to increase plasma concentration of rivaroxaban [79]. They are potential inhibitors of CYP2J2 but also strong CYP3A and P-gp/BCRP inhibitors. Therefore, it could also be valuable to measure the CYP2J2 activity in further studies assessing rivaroxaban exposure. Concerning apixaban, CYP2J2 and CYP1A2 contribute together to only 6% of the metabolism, and a significant impact of CYP2J2 is not expected [11]. Indeed, no study was found in the literature on the impact of CYP2J2 polymorphism on apixaban.

We found that renal insufficiency significantly increased the exposure to apixaban but not to rivaroxaban. This was unexpected, because all DOACs are eliminated by the kidneys [82]. It is thus largely accepted that impaired renal function directly influences the anticoagulation regimen [82]. Especially as rivaroxaban should logically be more impacted by renal dysfunction than apixaban, being 66% excreted by the kidney as compared to apixaban, which is only excreted at 25–30% [82,83].

One possible explanation for this finding is that normal and moderate renal functions were equally distributed in the apixaban cohort, whereas there were twice as many patients with normal renal function in the rivaroxaban cohort (Table 1).

Another explanation is a possibly inappropriate dosing regimen of apixaban and rivaroxaban according to creatinine clearance [84]. Rivaroxaban dose adjustment appeared to be more appropriate than apixaban dose adjustment according to renal function in our cohort, erasing the impact of this covariable. As shown in Table 1, adequate dosages (according to SmPC) were found to be prescribed in 70% of apixaban and 80% of rivaroxaban patients. This is consistent with existing real-world data that report off-label dosing of DOACs ranging from 13% to 57% [84,85]. Some studies published in the literature found that it was apixaban that had the highest rate of inappropriate dosing and others that it was rivaroxaban [84–86]. These rates seem dependent on the included population characteristics and could vary between studies [85]. In our study, we found a higher rate of inadequate dose selection with apixaban, which might be explained by the implication of other factors than renal function, such as age and weight [84]. Moreover, our consideration of both AF and venous thromboembolism (VTE), despite significant differences in terms of indication and dosing for VTE treatment, could be another explanation [84].

The multivariable linear regression models showed no effect of age, weight, gender, and ALAT on apixaban and rivaroxaban exposure when the phenotypic activity of CYP3A and P-gp were considered. In accordance with SmPC, gender is not a criterion for dose adjustment [8,9]. Moreover, recent population-based cohort studies did not find sex-related differences in terms of ADR occurrence or trough concentration levels of DOACs [87,88]. However, a prospective study in the perioperative setting found that female gender was a predictor for higher apixaban and rivaroxaban levels, with authors suggesting the cause being that females are at higher risk of renal insufficiency [89].

We observed an absence of association between age and apixaban PK parameters, even though it is a criterion for dose adjustment according to the SmPC [8,9]. However, our multivariable linear model found a tendency towards an increased apixaban exposure with an increase in age. The age effect could have been blurred, because most of our patients received an adequate dosage adjustment. Regarding the weight, only a small percentage of patients (16.46%) had a weight < 60 kg in our cohort, lowering the chance of finding a significant effect of this cofactor on exposure. In addition, weight and age have a small impact on exposure on their own. Indeed, subgroup analysis of the ARISTOTLE trial suggested that the presence of only one dose reduction criterion does not significantly impact the safety or efficacy [90]. Moreover, studies showed that patients with age > 65 years or weight < 50 kg were more exposed to apixaban, but there was no meaningful difference in clinical outcomes that would require dose modification based on age and weight alone [91,92]. In addition, a large register-based cohort study corroborated our results with no clear correlations found between BMI, age, and gender and trough concentrations of apixaban and rivaroxaban [88].

Phenotyping presents the advantage of measuring the effect of non-genetic factors, as compared to genotyping [93]. Indeed, even if the association between the SNPs and the enzyme activity is known, epigenetic and/or other factors can induce a phenoconversion [94–96]. Genotyping presents other limitations compared to phenotyping, such as the fact that functional consequences of most genetic polymorphisms have not yet been identified and that unknown/new SNPs cannot be tested [94,97,98]. Inter-individual variability in the PK of drugs due to genetic polymorphism has been identified, but our current knowledge does not allow any consistent predictions regarding patients' drug response [55,99]. Moreover, the influence of rare *ABCB1* variants on drug bioavailability and response has not been identified yet [55]. We tested the three most prevalent SNPs, but growing evidence suggests that rare variants might have greater effects on drug PK or PD than the more common ones [55]. Unexpectedly, we found that the CYP3A predicted activity from genotype was significantly associated with the $AUC_{0–6h}$ of rivaroxaban. However, as we found that the phenotype was not correlated to the genotype in our study, the

potential physiological meaning of this association remains unexplained. This underlined the difficulty of predicting the CYP3A phenotype from the genotype [96]. Each individual genetic factor associated to CYP3A expression has a minor role, because it is regulated by multiple genes, as suggested by its known continuous and unimodal distribution [73,81]. It is, therefore, expected to not find a strong association between CYP3A genetic polymorphisms and drug exposure. A phenoconversion may have occurred in IM patients due to various environmental factors. Indeed, more than three-quarters of included patients have an IM predicted phenotype from genotype in our cohort. The high proportion of IM could be explained by the fact that the CYP3A predicted phenotype from genotype considers both the CYP3A4 and the CYP3A5. Indeed, the CYP3A5*3 has a reduced activity and is present in 95% of the European and 62% of the whole population [47]. Therefore, the probability of having an intermediate CYP3A activity is high.

Overall, as previously shown, our results highlight the need to complete genotyping by phenotyping [96]. Indeed, it was shown that performing both tests simultaneously explained more clinical events than each of the tests being performed separately [96,98].

Our study has some limitations, such as the failure to reach the target sample size, which may have resulted in a lack of power and the lack of association with CYP3A. Additionally, the protocol did not plan to carry out a full PK sampling, and certain time points could be lacking, especially in the elimination phase. Using a phenotyping cocktail approach and MR as phenotypic metrics are subject to interpretation in terms of metabolizer classifications, but these metrics were used as continuous variables to address this concern. Another limitation is the inclusion of hospitalized patients exclusively, with all the variability in non-genetic factors that this implies. This could have led to CYP3A and P-gp activity and expression being influenced by heterogenous non-genetic factors, making it difficult to extrapolate our results to other populations, such as ambulatory or non-hospitalized patients.

5. Conclusions

In conclusion, our results indicate that P-gp phenotypic activity, rather than P-gp polymorphisms, has a relevant impact on the exposure of apixaban and rivaroxaban. Moreover, neither CYP3A phenotypic activity nor CYP3A predicted activity from genotype had a relevant impact on the exposure of these two DOACs.

Our study suggests that integrating P-gp phenotypic activity in the dose selection criteria may be beneficial. Genotyping of *CYP3A4*, *CYP3A5*, and *ABCB1* is probably not enough to predict enzyme activity due to the dynamic application of environmental, physiological, and pathophysiological factors. More studies are needed to assess the clinical utility of adding P-gp to dose selection in terms of adverse events and efficiency.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jpm12040526/s1, Figure S1. (a) $AUC_{0–6h}$ and (b) C_{2h} of rivaroxaban according to the CYP3A predicted activity from genotype; Table S1. SNPs of *CYP3A4* and *CYP3A5* studied; Table S2. Frequencies of the different genotypes found in our cohort as compared to frequencies found in reference population (Caucasian); Table S3. Multivariable linear regression models to assess if the phenotypic activity of CYP3A and P-gp are associated with the C_{2h} of apixaban and rivaroxaban. Each independent variable is reported with its beta coefficient (β) and its 95% confidence interval (CI95%).; Table S4. Multivariable linear regression models to assess if the genotype of CYP3A and P-gp are associated with the C_{2h} of apixaban and rivaroxaban. Each independent variable is reported with its beta coefficient (β) and its 95% confidence interval (CI95%); Table S5. Spearman's correlation between phenotype activity of CYP3A activity predicted by genotype and $MR_{midazolam}$ and between genotypes of P-gp and $AUC_{fexofenadine}$ for apixaban and rivaroxaban cohorts.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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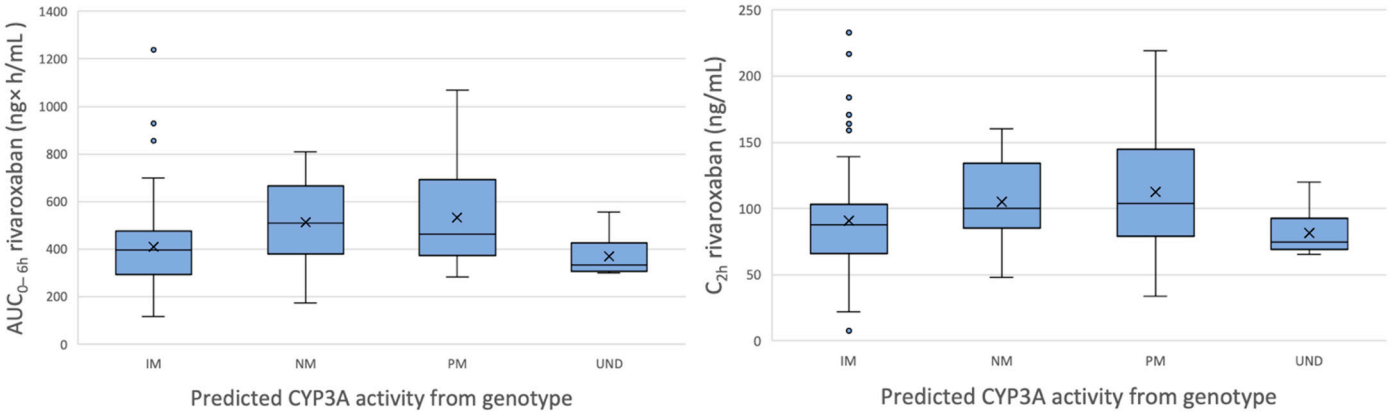
Supplementary Materials



(**a**) (**b**)

Figure S1. (**a**) AUC_{0-6h} and (**b**) C_{2h} of rivaroxaban according to the CYP3A predicted activity from genotype.

Table S1. SNPs of *CYP3A4* and *CYP3A5* studied.

Gene	rs Number	Common Allele Name
CYP3A4	rs12721629	CYP3A4*12,c.1117 C>T,g.21896C>T
	rs4987161	CYP3A4*17,c.566 T>C,g.15615T>C
	rs2740574	CYP3A4*1B, g.-392A>G
	rs55785340	CYP3A4*2,c.664T>C,g.15713T>C
	rs35599367	CYP3A4*22, g.15389C>T
	rs4986910	CYP3A4*3,c.1334T>C
CYP3A5	rs28365083	CYP3A5*2,g.27289C>A
	rs776746	CYP3A5*3/*10,g.6986A>G
	rs28383468	CYP3A5*3B,g.3705C>T
	rs10264272	CYP3A5*6,g.14690G>A
	rs41303343	CYP3A5*7,g.27131_27132insT
	rs55817950	CYP3A5*8,g.3699C>T
	rs28383479	CYP3A5*9,g.19386G>A

Table S2. Frequencies of the different genotypes found in our cohort as compared to frequencies found in reference population (Caucasian).

rs ID	Homozygous for Major Allele (Cohort)	Homozygous for Major Allele (Reference)	Heterozygous (Cohort)	Heterozygous (Reference)	Homozygous for Minor Allele (cohort)	Homozygous for Minor Allele (Reference)
rs10264272	0.993	0.994	0.007	0.006	0.000	0.000
rs12721629	1.000	1.000	0.000	0.000	0.000	0.000
rs2740574	0.936	0.946	0.064	0.052	0.000	0.002
rs28365083	0.993	0.992	0.007	0.008	0.000	0.000
rs28383468	0.969	0.978	0.031	0.020	0.000	0.002
rs28383479	1.000	1.000	0.000	0.000	0.000	0.000
rs35599367	0.892	0.903	0.105	0.095	0.003	0.002
rs41303343	1.000	1.000	0.000	0.000	0.000	0.000
rs4986910	0.980	0.986	0.017	0.014	0.003	0.000
rs4987161	1.000	1.000	0.000	0.000	0.000	0.000
rs55785340	0.997	0.996	0.000	0.0004	0.003	0.000
rs55817950	1.000	1.000	0.000	0.000	0.000	0.000
rs776746	0.881	0.891	0.112	0.105	0.007	0.004
rs1045642	0.264	0.266	0.458	0.503	0.278	0.231
rs2032582	0.311	0.316	0.451	0.489	0.239	0.159
rs1128503	0.315	0.334	0.468	0.501	0.217	0.165

Table S3. Multivariable linear regression models to assess if the phenotypic activity of CYP3A and P-gp are associated with the C_{2h} of apixaban and rivaroxaban. Each independent variable is reported with its beta coefficient (β) and its 95% confidence interval (CI95%).

	C_{2h} of Apixaban	**C_{2h} of Rivaroxaban**
Intercept	R^2	
	46%	22%
	−0.46 (−51.98 to 51.05); $p = 0.9859$	−77.74 (−161.1 to 5.63); $p = 0.0673$
Variables		
$MR_{midazolam}$, per log_{10}	6.07 (−8.08 to 20.22); $p = 0.3979$	−8.35 (−30.17 to 13.47); $p = 0.4503$
$AUC_{fexofenadine}$, per log_{10}	31.52 (14.92 to 48.13); $p = 0.0003$	47.19 (19.46 to 74.93); $p = 0.001$
Weight, per kg	−0.02 (−0.37 to 0.33); $p = 0.9097$	0.23 (−0.18 to 0.63); $p = 0.2713$
CrCl, per unit	−0.40 (−0.67 to −0.13); $p = 0.0042$	−0.017(−0.49 to 0.34); $p = 0.7212$
ALAT, per unit	0.06 (−0.13 to 0.25); $p = 0.5397$	0.05 (−0.14 to 0.23); $p = 0.6233$
Gender		
Male	Reference category	Reference category
Female	4.07 (−5.94 to 14.08); $p = 0.4233$	1.91 (−13.03 to 16.84); $p = 0.8009$
Dose		
2.5 mg bid	Reference category; $p < 0.0001$*	NA
5 mg bid	51.97 (41.16 to 62.78); $p < 0.0001$	NA
10 mg bid	116.28 (80.44 to 152.12); $p < 0.0001$	NA
10 mg od	NA	Reference category; $p = 0.0140$*
15 mg od	NA	18.95 (−21.77 to 59.66); $p = 0.3587$
20 mg od	NA	39.98 (−4.47 to 84.44); $p = 0.0775$
15 mg bid	NA	41.5 (0.24 to 82.75); $p = 0.0487$
Age		
<65 years	Reference category; $p = 0.4188$*	Reference category; $p = 0.2714$*
65–74 years	8.61 (−10.00 to 27.23); $p = 0.3619$	15.15 (−0.06 to 30.37); $p = 0.051$
75–84 years	13.69 (−5.59 to 32.97); $p = 0.1627$	10.55 (−12.65 to 33.75); $p = 0.3696$
>85 years	17.73 (−3.37 to 38.82); $p = 0.0990$	7.97 (−15.72 to 31.67); $p = 0.5066$

*p-value for the overall association between C_{2h} and the variable. Abbreviations: CrCl, creatinine clearance; ALAT, alanine transaminase; MR, metabolic ratio; AUC, area under the curve; C_{2h}, concentration 2 h after drug administration; bid, twice daily; od, once daily; NA, not applicable.

Table S4. Multivariable linear regression models to assess if the genotype of CYP3A and P-gp are associated with the C_{2h} of apixaban and rivaroxaban. Each independent variable is reported with its beta coefficient (β) and its 95% confidence interval (CI95%).

	C_{2h} of Apixaban	**C_{2h} of Rivaroxaban**
	R^2	
	39%	15%
Intercept	80.68 (42.43 to 118.93); $p = 0.0001$	−10.99 (−88.50 to 66.51); $p = 0.7792$
Variables		
Weight, per kg	0.01 (−0.38 to 0.36); $p = 0.9693$	0.46 (0.04 to 0.88); ***p* = 0.0328**
CrCl, per unit	−0.48 (−0.75 to −0.21); ***p* = 0.0006**	−0.14 (−0.61 to 0.32); $p = 0.5481$
ALAT, per unit	0.07 (−0.17 to 0.32); $p = 0.5684$	0.08 (−0.06 to 0.22); $p = 0.2719$
Predicted phenotype from genotype CYP3A		
IM	Reference category; $p = 0.1103$*	Reference category; ***p* = 0.0482***
NM	−2.16 (−23.45 to 19.13); $p = 0.8413$	20.74 (1.20 to 40.28); $p = 0.0378$
PM	−18.61 (−36.00 to −1.21); $p = 0.0362$	24.72 (−8.3 to 57.73); $p = 0.1407$
Genotype *ABCB1* 1236C>T		
No mutation	Reference category; $p = 0.7096$*	Reference category; $p = 0.4203$*
Heterozygous for mutation	−1.26 (−23.09 to 20.57); $p = 0.9093$	−16.43 (−46.63 to 13.77); $p = 0.2834$
Homozygous for mutation	−9.35 (−36.76 to 18.06); $p = 0.5013$	−1.63 (−39.30 to 36.03); $p = 0.9316$
Genotype *ABCB1* 3435C>T		
No mutation	Reference category; $p = 0.6778$*	Reference category; $p = 0.4836$*
Heterozygous for mutation	−7.52 (−24.64 to 9.60); $p = 0.3866$	−8.53 (−32.11 to 15.04); $p = 0.4747$
Homozygous for mutation	−2.70 (−18.04 to 12.65); $p = 0.7288$	−12.17 (−32.48 to 8.14); $p = 0.2375$
Genotype *ABCB1* 2677G>T		
No mutation	Reference category; $p = 0.7470$*	Reference category; $p = 0.3409$*
Heterozygous for mutation	8.08 (−14.86 to 31.01); $p = 0.4874$	23.36 (−8.85 to 55.56); $p = 0.1535$
Homozygous for mutation	9.94 (−17.81 to 37.69); $p = 0.4800$	16.54 (−19.70 to 52.77); $p = 0.3677$
Gender		
Male	Reference category	Reference category
Female	5.44 (−5.98 to 16.87); $p = 0.3206$	2.56 (−12.47 to 17.59); $p = 0.7361$
Dose		
2.5 mg bid	Reference category; ***p* < 0.0001***	NA
5 mg bid	51.17 (37.82 to 64.51); $p < 0.0001$	NA

10 mg bid	113.17 (81.73 to 145.52); $p < 0.0001$	NA
10 mg od	NA	Reference category; ***p* = 0.0002***
15 mg od	NA	29.53 (−2.56 to 61.63); $p = 0.0709$
20 mg od	NA	57.40 (22.52 to 92.28); $p = 0.0015$
15 mg bid	NA	60.99 (29.95 to 92.04); $p = 0.0002$
Age		
<65 years	Reference category; $p = 0.7224$*	Reference category; ***p* = 0.0284***
65–74 years	5.10 (−15.52 to 25.71); $p = 0.6257$	24.56 (8.41 to 40.71); $p = 0.0032$
75–84 years	9.29 (−10.54 to 29.12); $p = 0.3560$	25.66 (−1.56 to 52.88); $p = 0.0644$
>85 years	14.31 (−10.69 to 39.31); $p = 0.2597$	19.86 (−9.35 to 49.08); $p = 0.1806$

*p-value for the overall association between C_{2h} and the variable. Abbreviations: CrCl, creatinine clearance; ALAT, alanine transaminase; IM, intermediate metabolizer; NM, normal metabolizer, PM, poor metabolizer; bid, twice daily; od, once daily; NA, not applicable. Statistically significant values are marked with bold.

Table S5. Spearman's correlation between phenotype activity of CYP3A activity predicted by genotype and $MR_{midazolam}$ and between genotypes of P-gp and $AUC_{fexofenadine}$ for apixaban and rivaroxaban cohorts.

	Apixaban	**Rivaroxaban**
CYP3A activity predicted by genotype and $MR_{midazolam}$	ϱ = 0.123; ($p = 0.121$)	ϱ = 0.163; ($p = 0.065$)
Genotype of *ABCB1* 1236C>T and $AUC_{fexofenadine}$	ϱ = −0.050; ($p = 0.530$)	ϱ = −0.060; ($p = 0.496$)
Genotype of *ABCB1* 2677G>T and $AUC_{fexofenadine}$	ϱ = −0.011; ($p = 0.887$)	ϱ = 0.026; ($p = 0.772$)
Genotype of *ABCB1* 3435C>T and $AUC_{fexofenadine}$	ϱ = 0.013; ($p = 0.870$)	ϱ = −0.056; ($p = 0.528$)

Chapter 4: Impact of Acute Inflammation on Cytochromes P450 Activity Assessed by the Geneva Cocktail.

Summary

Pathophysiological factors and drug-disease interactions are one source of intra- and inter-individual variability in drug response, as explained in **chapter 1.** The impact of an environmental factor, through DDIs, on PK and PD profile of a drug was explored in **chapter 2**, and **chapter 4** aims thus to assess the influence of pathophysiological factors. Moreover, the study of the impact of pathophysiological factors and subsequent drug-disease interaction could help to interpret results found in **chapter 3**, because it concerns a hospitalized population.

Inflammation could be triggered by exogenous aggression, such as surgery, and thus lead to safety and efficacy issues of drugs by altering exposure. This may have an impact on new and existing treatments that should be adapted to transient inflammation to avoid under- or over-exposure.

The **research article 2** presented in **chapter 4** was published in *Clinical Pharmacology and Therapeutics*. It sustains the personalization of treatment as it aims to predict the impact of a drug-disease interaction by characterizing the impact of inflammation secondary to surgery on the six mains human CYPs. Thirty patients who underwent elective hip surgery were included in this prospective observational study. The MR of CYP1A2, 2B6, 2C9, 2C19, 2D6 and 3A were assessed by administering the Geneva cocktail before, one and three days after surgery and at discharge. The procedure of the phenotyping test was explained in **chapter 1**. To assess the intensity of inflammation, five biomarkers (IL-6, CRP, TNF-α, IL-1β, and interferon-gamma (IFN-γ)) were measured in patients' serum before, the first three days following surgery and at discharge. **Research article 2** showed that acute inflammation (hip surgery model) impacts on CYPs activities, with different direction, size and kinetics according to the isoforms considered. Indeed, CYP1A2, CYP2C19 and CYP3A activity decreased after surgery, while CYP2B6 and CYP2C9 activity increased. The maximal effects are isoform-specific in terms of amplitude and times. Surgery did not have a significant impact on CYP2D6 activity. The correlation with several variables such as pro-inflammatory markers levels, BMI, age, gender, smoking status, diabetes or DDIs were tested in a linear mixed model.

My contributions to the **research article 2** were the entire management of the clinical study, the recruitment of patients, the collection of samples and data, the assessment of the genotype, the analysis of the results and the article's writing.

Research article 2: Impact of Acute Inflammation on Cytochromes P450 Activity Assessed by the Geneva Cocktail.

Camille Lenoir, Youssef Daali, Victoria Rollason, François Curtin, Yvonne Gloor, Marija Bosilkovska, Bernhard Walder, Cem Gabay, Michael John Nissen, Jules Alexandre Desmeules, Didier Hannouche, Caroline Flora Samer.

Clinical Pharmacology & Therapeutics. 2021 Jun;109(6):1668-1676.

Impact of Acute Inflammation on Cytochromes P450 Activity Assessed by the Geneva Cocktail

Camille Lenoir[1,2], Youssef Daali[1,2], Victoria Rollason[1], François Curtin[1], Yvonne Gloor[1], Marija Bosilkovska[1], Bernhard Walder[3], Cem Gabay[4], Michael John Nissen[4], Jules Alexandre Desmeules[1,2], Didier Hannouche[5] and Caroline Flora Samer[1,*]

Cytochromes P450 (CYP) are subject to important interindividual variability in their activity due to genetic and environmental factors and some diseases. Limited human data support the idea that inflammation downregulates CYP activities. Our study aimed to evaluate the impact of orthopedic surgery (acute inflammation model) on the activity of six human CYP. This prospective observational study was conducted in 30 patients who underwent elective hip surgery at the Geneva University Hospitals in Switzerland. The Geneva phenotyping cocktail containing caffeine, bupropion, flurbiprofen, omeprazole, dextromethorphan, and midazolam as probe drugs respectively assessing CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A activities was administered orally before surgery, day 1 (D1) and 3 (D3) postsurgery and at discharge. Capillary blood samples were collected 2 hours after cocktail intake to assess metabolic ratios (MRs). Serum inflammatory markers (CRP, IL-6, IL-1β, TNF-α, and IFN-γ) were also measured in blood. CYP1A2 MRs decreased by 53% ($P < 0.0001$) between baseline and the nadir at D1. CYP2C19 and CYP3A activities (MRs) decreased by 57% ($P = 0.0002$) and 61% ($P < 0.0001$), respectively, with the nadir at D3. CYP2B6 and CYP2C9 MRs increased by 120% ($P < 0.0001$) and 79% ($P = 0.018$), respectively, and peaked at D1. Surgery did not have a significant impact on CYP2D6 MR. Hip surgery was a good acute inflammation model as CRP, IL-6, and TNF-α peak levels were reached between D1 and day 2 (D2). Acute inflammation modulated CYP activity in an isoform-specific manner, with different magnitudes and kinetics. Acute inflammation may thus have a clinically relevant impact on the pharmacokinetics of these CYP substrates.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ There is a high interindividual variability in cytochromes P450 (CYP) activities due to genetic and environmental factors, as well as some diseases. Limited human data supports the hypothesis that inflammation may downregulate CYP activities.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ What is the impact of acute inflammation triggered by elective hip surgery on the activity of the six major CYP isoforms in humans?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Acute inflammation (hip surgery model), had an impact on CYP activities in an isoform-specific manner, with different magnitudes and kinetics. Our results showed that patients who underwent hip surgery had lower activity of CYP1A2, CYP2C19, and CYP3A. In contrast, CYP2B6 and CYP2C9 activity increased after surgery, whereas variations in CYP2D6 activity were not significant for the duration of the study.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ A greater awareness of the impact of surgery on the pharmacokinetics of drugs metabolized by CYP could help improve drug efficacy and safety in the postoperative setting.

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Cytochromes P450 (CYP) are the major drug metabolic enzymes, predominantly expressed in the liver.[1] Among the 57 identified CYP, only a few contribute to drug metabolism with 6 isoforms, namely CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A, metabolizing 90% of marketed drugs.[1] The relative importance of the clearance mechanisms mediated by these isoenzymes range from 46% carried out by members of the CYP3A family, to 16% by CYP2C9, 12% by CYP2C19 and 2D6, 9% by CYP1A, and 2% by CYP2B6.[1] Interindividual variability in CYP activity has been observed as a result of genetic and environmental factors or different disease states.[1]

Genetic polymorphism and/or drug interactions (CYP inhibitors or inducers) can markedly alter drug response, with potential adverse drug reactions (ADRs) and even contribute to the removal of drugs from the market because of unexpected ADR.[1] The ADRs are the fourth leading cause of death in the United States.[2] They trigger hospitalizations or extend hospital stay, whereas being probably preventable in up to three quarters of cases.[2]

Data are further accumulating to point out that the activity of most of the CYPs can either increase or decrease in the presence of endogenous substances, such as proinflammatory cytokines, which can also lead to pharmacokinetic changes and significant drug-drug interactions. Cytokines are intercellular messengers that play a critical role in mediating inflammatory responses and can be additive, synergistic, or inhibitory with each other.[3] Interleukin (IL)-6 is a prototypic proinflammatory cytokine that is directly associated with the degree of inflammation and tissue injury.[4]

Data from *in vitro* and animal models as well as more limited human data support the hypothesis that inflammatory responses are associated with significant reduction in CYP activities.[5] This may alter hepatic clearance of drugs not limited by blood flow.[6] Several mechanisms have been proposed to explain CYP activities' modulation by acute and chronic inflammatory states but the predominant one involves CYP gene expression downregulation by proinflammatory cytokines, such as IL-1, IL-6, and tumor necrosis factor (TNF)-α.[7]

In vitro and animal studies have demonstrated CYP3A downregulation with reduction of mRNA levels.[6,8] In rodents, an acute inflammatory response is associated with a decrease in CYP3A11 mRNA hepatic expression and the causative role of each individual cytokine in CYP3A repression has been studied.[6,8] Moreover, in human hepatocytes cultures, the inducible expression of CYP3A by rifampicin was shown to be suppressed by IL-6.[6,8] In humans, CYP3A activity reduction was maximal 3 days postsurgery with a decrease of 20–60% from baseline levels, depending on the type of surgery.[9] Furthermore, a negative correlation was observed between CYP3A activity and IL-6 peak levels ($r_s = -0.54$, $P = 0.03$).[9] A prospective study in 40 patients with biopsy-proven advanced malignancies showed that the acute-phase response as assessed by C-reactive protein (CRP) levels > 10 mg/L was associated with an average 30% reduction of CYP3A4 activity ($P = 0.0062$).[10] However, the area under the curve (AUC) of atorvastatin, a CYP3A4 substrate, was not modified by cardiac surgery.[11]

The mechanism by which CYP3A gene expression is downregulated by cytokines suggests that the activity of other CYPs could be similarly modulated. Indeed, a key factor appears to be the interplay between inflammatory signaling pathways and transcription factors.[12] Different mediators and transcription factors have been shown to be involved in the regulation of different CYP genes, such as NF-κB, AP-1, SP-1, CAR, PXR, TLR-4, CCAAT enhancer binding proteins family, hepatocyte nuclear factor, and signal transducer and activator of transcription families.[12]

Three case reports describe patients stable on clozapine therapy who developed clozapine toxicity due to increased clozapine plasma concentrations after an infection and/or an inflammatory process, such as surgery, which may be related to cytokine-mediated inhibition of CYP1A2.[5]

Finally, antipyrine (CYP1A2, 2B6, 2C, and CYP3A substrate) and meperidine (CYP3A substrate) plasma half-lives were both significantly decreased during the acute phase of hepatitis compared with recovery period or healthy subjects, although part of the effect might be caused by liver damage among others.[13,14]

The main clinical consequence and concern of these findings is that an inflammatory process can modify exposure to a previously stable drug regimen, thereby possibly resulting in either an increased incidence of ADRs or a lack of efficacy.[6] We therefore sought to evaluate the effects of elective hip surgery as a model of acute inflammation on the activity of the six major CYPs in hospitalized patients using a phenotyping cocktail approach. Total hip surgery was chosen as a model for acute inflammation as it is known to be associated with a significant inflammatory response.[15]

METHODS

Study protocol

This study was a prospective open label observational study investigating the impact of elective hip surgery on the activities of 6 major CYPs, namely CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A. Study protocol (No. 2016-02232) was approved by the regional research ethics committee of the canton of Geneva (CCER) and registered on the US National Institutes of Health clinical trials registry (NCT03262051). Written informed consent was obtained from all patients prior to initiation of any study procedure. This clinical trial was carried out in compliance with the principles of the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice Guidelines.

Study population

Participants were recruited during the pre-operative anesthesia visit for an elective hip surgery scheduled at the Geneva University Hospitals, over a period of 16 months. Eligible patients underwent an elective surgery for hip osteoarthritis and were older than 18 years of age. Exclusion criteria included pregnancy, breastfeeding, and allergy to any of the components of the Geneva cocktail (caffeine, flurbiprofen, omeprazole, bupropion, dextromethorphan, fexofenadine, and midazolam) as well as severe cardiac failure, severe edema or ascites, severe chronic obstructive pulmonary disease or pulmonary embolism requiring oxygen, renal impairment (defined as serum creatinine concentrations > 1.5 × upper limit normal), hepatic impairment (defined as transaminases, bilirubin, gamma glutamyl transferase > 2 × upper limit normal), HIV infection, active cancer, uncontrolled infection, or inflammatory arthritis. Moreover, comedications were systematically screened and patients taking CYP inhibitors or inducers were excluded, using the Lexi-Interact drug interaction checker and the Geneva table of CYP substrates, inhibitors, and inducers.[16,17] Proton pump inhibitor use was allowed in the postoperative setting, as it is a routine prescription after surgery in our hospital that could thus not be excluded. Esomeprazole was the only

proton pump inhibitor administered to the study subjects. The linear mixed model was thus adjusted for esomeprazole intake as it is a well-known CYP2C19 inhibitor.

The primary objective was to measure the variation in the activity of six major CYPs post hip surgery.

Genotyping of CYP2D6, CYP2B6, CYP2C9, and CYP2C19

The method has previously been described in detail in the literature.[18] Briefly, genomic DNA was extracted from EDTA whole blood samples using the QIAamp DNA blood mini kit (Qiagen, Hombrechtikon, Switzerland). Genotyping was performed using TaqMan OpenArray genotyping assays (Life Technologies Corporation, Carlsbad, CA) on a QuantStudio 12K Flex Real-time PCR System (Thermo Fisher Scientific, Rochester, NY). Single-nucleotide polymorphisms used to assess the CYP genotype are listed in **Table S1**. CYP2D6 gene duplication were also assessed with the TaqMan Copy Number Assay Hs00010001 with RNase P as references (Thermo Fisher Scientific). AlleleTyper Software (Thermo Fisher Scientific) was used to translate genetic pattern information from genotyping (Single-nucleotide polymorphisms) and copy number assay to pharmacogenomic gene-level star (*) nomenclature. Translational tables (Thermo Fisher Scientific and PharmGKB) were used to determine genotype for each CYP (star allele nomenclature).

Phenotyping

The metabolic ratio (MR) of 6 CYPs (1A2, 2B6, 2C9, 2C19, 2D6, and 3A) was measured before surgery (D0), day 1 (D1) and day 3 (D3) after surgery and at discharge. Phenotype assessment was performed using the orally administrated probe substrates contained in the Geneva cocktail (caffeine 50 mg, CYP1A2; bupropion 20 mg, CYP2B6; flurbiprofen 10 mg, CYP2C9; omeprazole 10 mg, CYP2C19; dextromethorphan 10 mg, CYP2D6, and midazolam 1 mg, CYP3A). The absence of mutual drug-drug interactions within the Geneva cocktail was previously demonstrated and bupropion is used at such a low dose that no effect on CYP2D6 activity is demonstrated.[19] The cocktail was also previously validated using dried blood spots as a sampling method.[20] Capillary blood samples were collected 2 hours after drug administration in a fasting patient and dried blood spots were stored at –20°C in a sealable plastic bag until analysis, as previously described.[21]

Phenotypic classification was based on MR (defined as the concentration of the metabolite divided by the concentration of the substrate), according to a validated method using liquid chromatography tandem mass spectrometry quantification.[20,22,23] Patients were classified as poor metabolizers (PMs), normal metabolizers (NMs), and ultra-rapid metabolizers (UMs) according to their MRs, as well as intermediate metabolizers for CYP2D6. Threshold values used for phenotype assessment are detailed in **Table S2**.[20,21]

Inflammatory marker levels

Serum levels of IL-6, CRP, TNF-α, IL-1β, and IFN-γ were measured early in the morning, prior to surgery (D0), the first 3 days postsurgery (D1, D2, and D3), and at discharge. The routine concentrations of CRP were measured from lithium heparin whole blood sample, directly after blood collection using latex enhanced immunoturbidimetry. Blood samples underwent centrifugation at 2,000 g and 4°C for 10 minutes and serum samples were stored at –80°C until analysis. Cytokines serum levels were measured using a validated Fluorokine MAP Cytokine Multiplex Elisa assay.

Statistical analysis

A sample size of 30 subjects was required in order to detect a difference of 30% in CYP activity with a power of 80% and an α-value of 5%. All statistical analyses were performed using the IBM SPSS Statistics software version 25 (Chicago, IL) and a *P*-value < 0.05 was considered as statistically significant. Means ± SDs were used to describe continuous variables.

Table 1 Mean MRs ± SD of the six CYP isoforms during the study course (baseline, D1, D2, D3, and discharge; *n* = 30

Isoform	MRs parameters ([Mean] ± SD)	D0	D1	*P* value	D3	*P* value	Discharge	*P* value
CYP1A2	[paraxanthine]/[caffeine]	0.406 ± 0.174	0.190 ± 0.095	< 0.0001	0.207 ± 0.075	< 0.0001	0.264 ± 0.111	0.106
CYP2B6	[OH-bupropion]/[bupropion]	1.591 ± 1.069	3.501 ± 1.613	< 0.0001	3.728 ± 2.309	< 0.0001	4,199 ± 1.988	0.002
CYP2C9	[OH-flurbiprofen]/[flurbiprofen]	0.043 ± 0.021	0.077 ± 0.077	0.018	0.057 ± 0.026	0.002	0.063 ± 0.036	0.007
CYP2C19	[OH-omeprazole]/[omeprazole]	0.760 ± 0.485	0.688 ± 0.745	0.488	0.324 ± 0.509	0.0002	0.564 ± 0.720	0.085
CYP2D6	[dextrorphan]/[dextromethorphan]	1.217 ± 1.459	0.902 ± 0.981	0.334	0.946 ± 1.063	0.330	0.608 ± 0.509	0.062
CYP3A	[OH-midazolam]/[midazolam]	0.888 ± 0.539	0.797 ± 0.359	0.252	0.337 ± 0.125	< 0.0001	0.336 ± 0.095	0.0001

P-values were calculated in comparison with baseline.
D, day; MRs, metabolic ratios.

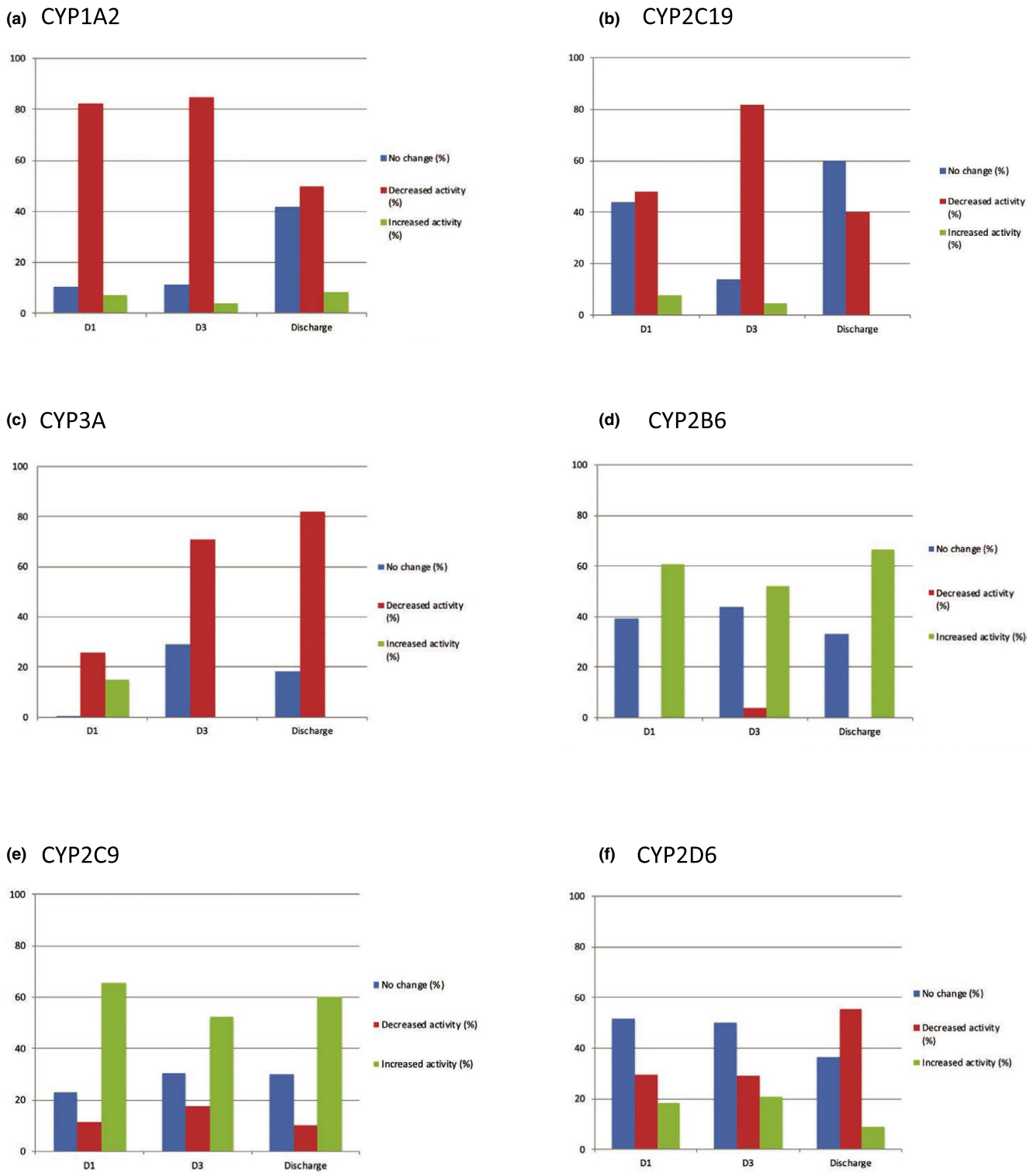

Figure 1 Percentage of patients (n = 30) demonstrating CYP phenoconversion at day (D)1, D3, and discharge: (**a**) CYP1A2, (**b**) CYP2C19, (**c**) CYP3A, (**d**) CYP2B6, (**e**) CYP2C9, and (**f**) CYP2D6

Comparisons of MRs and levels of inflammatory markers before and after surgery were expressed in percentages and analyzed using a paired t-test.

Spearman's rank correlations were used to assess correlation between CYP MRs and inflammatory markers levels, as well as gender, age, body mass index (BMI), or length of surgery. A linear mixed model was built taking into account the repetition of measurements in the same patients as a function of time, to assess the factors (covariables) influencing CYP activities (dependent variables), such as inflammatory markers, BMI, age (continuous variables), as well as surgery, gender, esomeprazole intake, or smoking status (binary variables).

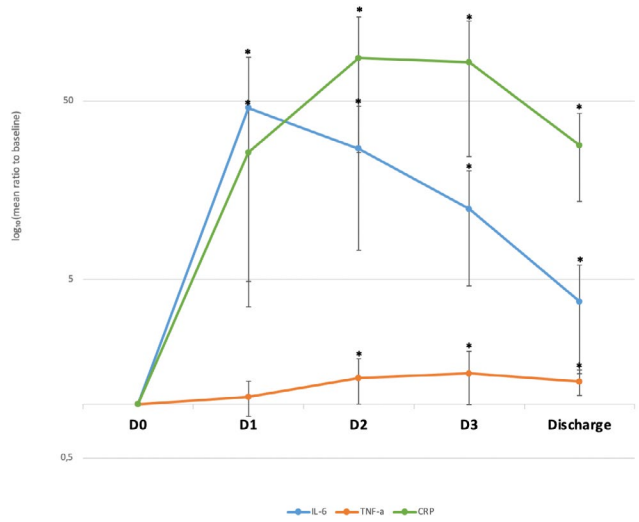

Figure 2 Log_{10} ratio to baseline levels of CRP, IL-6, and TNF-α at baseline, day (D)1, D2, D3, and discharge (n = 30). Error bars represent SD. The P-values were calculated in comparison with baseline, *P < 0.05

RESULTS

Demographic

Thirty White subjects were included with a mean age of 68 ± 11 years and BMI of 27 ± 6. Eighteen subjects (60%) were women. Two patients with type II diabetes were included. The mean duration of surgery was 91 ± 34 minutes, ranging from 54 to 220 minutes. The mean hospital duration after surgery was 4 ± 1 day, ranging from 2 to 6 days. None of the subjects had any drug safety concerns.

CYP activity before and after surgery

The activities of the 6 major CYPs before and after surgery are reported in **Table 1**. CYP1A2 MRs decreased by 53.2% (P < 0.0001), with a maximal effect at D1 postsurgery. CYP2C19 and CYP3A activities decreased by 57.5% (P = 0.0002) and 61.3% (P < 0.0001), respectively, between baseline and the nadir at D3 postsurgery. Conversely, CYP2B6 and CYP2C9 MRs increased by 120.1% (P < 0.0001) and 79.1% (P = 0.018), respectively, and were maximal at D1. The decrease of CYP2D6 MRs (50.0%) did not reach statistical significance before discharge (P = 0.062). None of the MRs of the six CYPs returned to normal levels prior to discharge.

Phenoconversion

All patients were genotyped and allelic frequencies for each CYP studied are presented in **Table S3** with predicted phenotypes.

The phenoconversion of CYP1A2, CYP2C19, CYP2D6, and CYP3A was assessed in phenotypic non-PM subjects after surgery. The phenotypic switch after surgery from NM to PM or from UM to NM was seen in 82% of subjects for CYP1A2 and CYP2C19 and 70% for CYP3A4 (**Figure 1a–c**). Concerning CYP2B6 and CYP2C9, as the MRs increased after surgery, UM subjects were excluded from the analysis. Sixty percent and 65% of patients had a phenotypic switch from either PM to NM or NM to UM, respectively (**Figure 1d,e**). Regarding CYP2D6, 55% of patients had a phenotypic switch at discharge (NM to intermediate metabolizer; **Figure 1f**).

Proinflammatory markers

The effects of surgery on inflammatory markers (CRP, IL-6, and TNF-α) exposure are shown in **Figure 2**. IL-6 serum levels peaked at D1, whereas TNF-α and CRP peaked at D2 postsurgery. IL-1β and IFN-γ were undetectable.

Circulating levels of TNF-α correlated with CRP (r = 0.542, P = 0.001) and IL-6 (r = 0.435, P = 0.013) levels. As expected, the correlation between circulating levels of IL-6 and CRP was even stronger (r = 0.613, P = 0.0001).

No correlation was demonstrated with gender, age, or BMI (P > 0.05 for all). Serum levels of IL-6 correlated with duration of hip surgery (r = 0.433, P = 0.017).

Variables that influenced change in CYP activity

No statistically significant correlation was demonstrated between extreme CYP MRs and peak levels of inflammatory markers.

Table 2 shows the correlation between MRs of each CYP isoforms and corresponding IL-6, TNF-α, and CRP serum levels.

A linear mixed model was built to assess the factors correlated with CYP activities, such as inflammatory markers, BMI, gender, age, esomeprazole intake, or smoking status (**Table 3**).

Several variables were significantly correlated with the activity of some CYPs, such as surgery (CYP1A2, 2B6, 2C9, and 3A), CRP (CYP2C19 and CYP3A), IL-6 (CYP3A), BMI (CYP1A2 and 2C19), and esomeprazole intake (CYP2C19). Age, gender, ethnicity, and smoking status were not correlated with CYP variations.

DISCUSSION

We assessed the impact of acute inflammation (elective hip surgery) on the activity of six major CYPs and demonstrated that surgery modulated CYP activity in an isoform-specific manner, with different magnitudes and kinetics. To our knowledge, this is the first time that CYP activities, other than CYP3A, have been studied in the postoperative setting.

In our study, CYP3A activity decreased by 60% after surgery (maximal after 3 days) and was inversely correlated with surgery and CRP, and positively correlated with IL-6. Previous publications have demonstrated that infection and more broadly inflammation decreased CYP3A activity, and in proportion to the severity of the disease.[5,11,14,24,25] Moreover, authors have shown that CYP3A4 activity was inversely correlated to CRP levels.[26,27] Surgery and cancer have also been associated with decreased CYP3A4 activity and increased serum levels of CRP and IL-6, respectively.[9,10] Moreover, in patients with rheumatoid arthritis, when inflammation was reversed by tocilizumab, an anti-IL-6 receptor antibody, exposure to simvastatin was significantly reduced by half at 1 and 5 weeks after infusion.[5] This is in line with our findings regarding CRP but not IL-6. Comparison of the correlation between CYP activities and IL-6 both before and after inflammation was not assessed in most published studies. A direct correlation over a short period of time would not be necessarily expected, because a time lag between IL-6 levels elevation

Table 2 Correlation (Spearman) among the MRs of the six CYP isoforms and IL-6, TNF-α, and CRP serum levels measured at specific timepoints in the 30 subjects

	CYP1A2	CYP2C19	CYP3A	CYP2B6	CYP2C9	CYP2D6
IL-6	−0.517 *P = 0.0001*	−0.165 *P = 0.102*	0.022 *P = 0.828*	0.336 *P = 0.001*	0.347 *P = 0.001*	−0.127 *P = 0.209*
CRP	−0.400 *P = 0.0001*	−0.417 *P = 0.0001*	−0.527 *P = 0.0001*	0.447 *P = 0.0001*	0.172 *P = 0.088*	−0.136 *P = 0.180*
TNF-α	0.135 *P = 0.183*	−0.104 *P = 0.308*	−0.296 *P = 0.003*	0.002 *P = 0.985*	−0.009 *P = 0.927*	−0.257 *P = 0.010*

and CYP downregulation could be expected. A 3-day lag after surgery between IL-6 elevation and CYP3A downregulation has already been described.[9] Furthermore, the mean IL-6 peak levels in our study were 1.6-fold to 5.1-fold lower than those previously reported in other types of surgery (peripheral vascular surgery with graft and abdominal aortic aneurysm, respectively).[9] Further investigations would be needed to confirm our results after cardiovascular surgery. If confirmed, other preclinical experiments would be required to understand the pathophysiology behind the association between CRP levels and CYP3A activity using *in vitro* and animal models.

Similarly to our results, many studies found decreased CYP1A2 activity in inflammatory conditions, such as infection or induced-infection models.[5,28] Even though tobacco smoking is a known inducer of CYP1A2, we did not find that smokers' status modulated CYP1A2 activity in our study, probably because of the small number of smokers ($n = 6$) and as smoking is forbidden in the hospital setting.[5] Significant inverse associations have previously been established between IL-6 levels and CYP1A2 activity ($r = -0.5$, $P = 0.0235$) but not with TNF-α, in 16 patients with congestive heart failure.[29] Several case reports have described increased clozapine toxicity or plasma concentration after infection and/or inflammatory processes.[5] The decrease of CYP1A2 activity described in our study confirms that it could be of clinical relevance as a phenoconversion was seen in 82% of patients. These changes in CYP1A2 activity led to increased risk of ADR and required dose adaptation.[30] Some authors reported an association between circulating concentrations of CRP and clozapine.[30,31] These published studies are in agreement with our results, because we found an inverse Spearman's correlation with IL-6 and CRP but not with

Table 3 Standardized variables in the linear mixed model and correlation with the metabolic activity of the six CYP isoforms in the 30 subjects

	CYP1A2	CYP2C19	CYP3A	CYP2B6	CYP2C9	CYP2D6
Surgery	−1.1867 (SE = 0.2215) *P* = 0.0001	0.4685 (SE = 0.2941) *P* = 0.115	−0.5622 (SE = 0.2079) *P* = 0.008	1.1910 (SE = 0.2117) *P* = 0.0001	0.6516 (SE = 0.2699) *P* = 0.018	−0.2428 (SE = 0.1842) *P* = 0.192
IL-6	−0.0935 (SE = 0.0863) *P* = 0.282	0.1004 (SE = 0.0914) *P* = 0.275	0.2902 (SE = 0.0809) *P* = 0.001	−0.1041 (SE = 0.0816) *P* = 0.206	0.0611 (SE = 0.1053) *P* = 0.563	−0.0349 (SE = 0.0700) *P* = 0.619
CRP	−0.0990 (SE = 0.0999) *P* = 0.324	−0.3045 (SE = 0.1062) *P* = 0.005	−0.2757 (SE = 0.0965) *P* = 0.005	−0.0295 (SE = 0.0970) *P* = 0.762	−0.1519 (SE = 0.1220) *P* = 0.216	0.0748 (SE = 0.0879) *P* = 0.398
TNF-α	0.1278 (SE = 0.0977) *P* = 0.198	0.1779 (SE = 0.1136) *P* = 0.123	−0.0333 (SE = 0.1113) *P* = 0.766	−0.0903 (SE = 0.1144) *P* = 0.432	−0.0727 (SE = 0.1206) *P* = 0.549	−0.1826 (SE = 0.1133) *P* = 0.111
BMI	0.2157 (SE = 0.1049) *P* = 0.049	−0.4965 (SE = 0.1261) *P* = 0.0001	−0.1768 (SE = 0.1345) *P* = 0.201	−0.0960 (SE = 0.1514) *P* = 0.531	0.2444 (SE = 0.0011) *P* = 0.056	0.0279 (SE = 0.1997) *P* = 0.890
Age	0.06678 (SE = 0.0962) *P* = 0.493	−0.2008 (SE = 0.1205) *P* = 0.106	0.0393 (SE = 0.1281) *P* = 0.761	−0.0754 (SE = 0.1432) *P* = 0.602	−0.0475 (SE = 0.1192) *P* = 0.693	−0.0432 (SE = 0.1869) *P* = 0.819
Gender (male)	0.0787 (SE = 0.1854) *P* = 0.674	0.0867 (SE = 0.2319) *P* = 0.712	−0.3386 (SE = 0.2530) *P* = 0.194	−0.1041 (SE = 0.2883) *P* = 0.721	0.1157 (SE = 0.2300) *P* = 0.618	−0.2868 (SE = 0.3817) *P* = 0.460
No intake of esomeprazole	n.a.	0.7763 (SE = 0.2737) *P* = 0.006	n.a.	n.a.	n.a.	n.a.
Nonsmoker	−0.1089 (SE = 0.2278) *P* = 0.636	n.a.	n.a.	n.a.	n.a.	n.a.

BMI, body mass index; MRs, metabolic ratios; n.a., not applicable.

TNF-α. However, conflicting results were reported in patients with diabetes.[32,33] In our study, only surgery was inversely correlated with CYP1A2 activity in the linear mixed model, but not cytokines' levels. This means that surgery triggered changes, other than an increase in cytokines' levels that could be responsible for the downregulation of CYP1A2 activity. It is indeed well-known that CYP1A2 is easily modulated by endogenous compounds and xenobiotics. BMI was also positively correlated to CYP1A2 activity in our study, but at the limit of significance. This has never been shown before in the literature.

We demonstrated that CRP was inversely correlated to CYP2C19 MR but that surgery, IL-6, and TNF-α were not. Other possible changes caused by surgery are therefore not involved in the downregulation of CYP2C19 activity. In patients with type 2 diabetes, CYP2C19 activity significantly decreased by half ($P = 0.001$) as compared with controls and multivariate models showed that IFN-γ and TNF-α partly explained these variations.[32] Moreover, CRP and IL-6 were significantly and inversely associated with CYP2C19 activity.[29,34] Other authors showed that CYP2C19 predicted and measured phenotype in patients with cancer were statistically discordant, but no significant correlations between the levels of any individual cytokine (CRP, IL-1β, IL-1α, IL-6, TNF-α, and TGF-β) were found.[5]

In our study, BMI was associated with a significant CYP2C19 activity reduction, which is supported by the literature.[35,36] In fact, the rate of high on-treatment platelet reactivity to clopidogrel was significantly associated with higher BMI as well as CYP2C19 loss-of-function alleles (LoFAs) carrier (*2 or *3).[35] In LoFA noncarriers with overweight/obesity, clopidogrel-aspirin therapy was not efficient in reducing the risk of stroke recurrence as compared with LoFA noncarriers with low/normal weight.[36] Again, we expect CYP2C19 activity decrease to be clinically relevant due to the observed phenoconversion in 82% of patients.

In the literature, it is described that cytokines downregulate CYP activity and this is consistent with our results, because we have shown that it is not the increase in cytokines' levels that is responsible of induction of CYP2B6 and 2C9 activities, but other mechanisms induced by surgery. Indeed, surgery was positively correlated to CYP2B6 and 2C9 MRs in our study and not to IL-6, CRP, and TNF-α levels.

We showed that CYP2B6 activity increased from the first day after surgery and that cytokine levels were not correlated to CYP2B6 MR when the model was adjusted to surgery status. Published data rather reported CYP2B6 activity decrease in inflammatory conditions.[32,37] A multivariate model conducted in patients with type II diabetes showed that IFN-γ and TNF-α partly explained these variations and the administration of IFN-α before cyclophosphamide (CP) caused a 63% decrease in its clearance ($P = 0.004$) compared with 24 hours after CP.[32,37] However, CP is a prodrug bioactivated by both CYP3A4 and 2B6.[37] The contribution of decreased CYP3A activity could thus not be ruled out. Hepatic CYP2B genes represent the most inducible CYP isoforms by phenobarbital-type compounds in most mammalian species.[38]

Phenoconversion was observed in 60% of our cohort of patients. One of the major factors that contribute to CYP2B6 modulation, like other inducible CYP, is the regulation of its transcription by several nuclear hormone receptors, such as PXR, CAR, glucocorticoid receptor (GR), and vitamin D receptor, in a direct and/or indirect manner.[38] In addition, CYP2B6 expression is inducible under stress conditions, such as fasting or energy restriction.[38] As cortisol, the glucocorticoid "stress hormone" binds the GRs, and increases under stress conditions, such as surgery, induction of CYP2B6 by surgery itself via the GR cannot be excluded.[39] In a randomized controlled study conducted in patients with elective hip surgery, cortisol levels indeed changed over time ($P < 0.001$).[40] The GR could also be implicated in CYP2C9 induction.[41]

We established that CYP2C9 activity increased after surgery, and was correlated with IL-6 but not with CRP and TNF-α. Several studies confirmed that the activity of CYP2C9 increased under inflammatory conditions as a consequence of a disease state or exogenous administration of cytokines.[5,32] However, conflicting results have been published, in particular with warfarin and losartan, where increased plasma concentration or bleeding events were reported during inflammation.[5,42,43] Nevertheless, warfarin and losartan are mainly metabolized by CYP2C9, but are also minor substrates of CYP3A4, 2C19, 1A2, and CYP3A, respectively, whose activities were reduced in our study. Moreover, the increase of CYP2C9 activity found in our study could be considered as clinically relevant as phenoconversion was seen in 65% of patients.

We described that CYP2D6 activity did not change significantly in the first 3 days after surgery, but a trend for a 50% decrease was noted at discharge, and inversely correlated with surgery and TNF-α levels. Other authors have also suggested that acute inflammation does not impact on CYP2D6 activity, as well as diabetes (type I, type II, and gestational).[32,33,44–46] In a study conducted in patients with congestive heart failure, TNF-α and IL-6 levels were furthermore not associated with CYP2D6 activity.[29] However, another study showed that CYP2D6 activity (mean urinary dextromethorphan ratio for 4 consecutive days) was significantly higher in HIV-infected patients than in healthy volunteers.[5] Thus, a decrease of CYP2D6 activity could occur at a later stage than that of other isoenzymes and this would be in line with our results where CYP2D6 activity decreased by 50% at discharge. Phenoconversion of CYP2D6 was observed in 55% of our cohort. The clinical relevance of this finding remains to be demonstrated due to the wide variability of CYP2D6 activity.

Three patients were CYP2D6 genotypic PMs in our study, and they were kept in our analysis because the correlation with CYP2D6 MRs were overall not significantly different whether they were included or not in the analysis. Besides, the genotypic activity of their other CYP was normal.

We carefully reviewed the anesthetics and analgesics administrated during the peri-operative period in order to exclude an impact on the activity of CYP, on top of the comedications systematically screened before surgery (exclusion of CYP inhibitors or inducers). None of the anesthetics and analgesics used were known to modulate CYP activity, except for propofol that has been shown to be weak CYP3A inhibitor, mainly

in *in vitro* studies. A double-blind randomized study conducted in 24 patients showed that the impact on midazolam metabolite formation was only statistically significant during the first 30 minutes of anesthesia induction with propofol but not during the 6 hours thereafter,[47] due to the short half-life of the molecule. It is therefore reasonable to exclude a significant impact of these medications administrated in the peri-operative setting on the activity of assessed CYP.

We thus showed that surgery had an impact on CYP in an isoform-specific manner that may have a clinically relevant impact on regular treatment and analgesia after surgery, such as CYP3A, CYP2C9, CYP2C19, and CYP1A2 substrates. In our study, more than 50% of patients were receiving CYP substrate to treat comorbidities and among analgesic drugs, almost three quarters were CYP substrates. Furthermore, these variations in MRs were of different magnitudes and kinetics and were correlated with different inflammatory markers.

Events, such as surgery, trauma, infection, burns, or advanced cancer, have been associated with significant variations in plasma concentration of acute phase proteins.[48] IL-6 cytokine is the key stimulator of acute phase protein production as well as other cytokines, such as IL-1β, TNF-α, and IFN-γ. Moreover, TNF-α and IL-6 promote the transcriptional induction of the CRP gene.[49] This supports our finding of a correlation among IL-6, TNF-α, and CRP. The modest effect of TNF-α found in our study might thus be an indirect effect of IL-6.

Different factors have been shown to influence systemic cytokine levels and some cytokines have extremely brief half-lives, making their detection difficult. In fact IL-1β and IFN-γ are rarely detectable in human serum, except in the case of severe inflammation or after intensive sampling in the perioperative period.[50,51] Authors have shown that IL-6 peak levels were reached 4–48 hours after surgery and fell rapidly after 48–72 hours.[51] CRP levels appear to rise more slowly postoperatively compared with cytokine levels.[52] In a study conducted in the same conditions as ours, CRP level reached its peak 2 days after surgery and remained significantly elevated on the third day after surgery.[15] These findings are in line with our study data.

Similarly to our results, other authors have found a correlation between increased IL-6 levels and the duration of surgery but not with gender after elective hip surgery.[53] Cytokine levels have been shown to increase with age, but we only observed a trend for IL-6 and TNF-α.[54] In accordance with our results, no correlation was found in the literature between cytokine levels and either BMI or gender.[55]

Our study has some limitations. The sample size was relatively small and confirmation of our linear mixed model findings in an additional and/or larger sample is warranted. Moreover, only two patients with type II diabetes were included in our cohort and it was thus impossible to draw any conclusion on the impact of type II diabetes on CYP activities. Furthermore, due to the methodology and statistical analyses used, a correlation between surgery and modulation of CYP activity was shown, but further investigations are needed to strengthen criteria of causation.

To conclude, our results indicate that surgery and acute inflammation have a major impact on the activity of six major CYPs in an isoform-specific manner of different magnitude and velocity. Our findings could thus have a relevant impact on the pharmacokinetics of drugs metabolized by these key drug-metabolizing enzymes and could help improve drug efficacy and safety in the postoperative setting.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHORS CONTRIBUTIONS

C.L., V.R., and C.F.S. wrote the manuscript. Y.D., Y.G., M.B., B.W., D.H., C.F.S., C.G., M.J.N., and J.A.D designed the research. C.L. performed the research. C.L., F.C., and C.F.S. analyzed the data. C.L., Y.D., and Y.G. contributed new reagents/analytical tools.

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Table S1: SNP polymorphisms of CYP2B6, 2C9, 2C19 and 2D6 assessed in the study.

Gene	rs number
CYP2B6	rs28399499 rs34223104 rs3211371
CYP2C9	rs28371685 rs1799853 rs1057910 rs56165452 rs28371686 hCV32287221
CYP2C19	rs6413438 rs12248560 rs4244285 rs4986893 rs28399504 rs56337013 rs72552267 rs72558186 rs41291556 rs17884712
CYP2D6	rs16947 rs1135840 rs5030862 rs5030865 rs28371706 rs59421388 rs35742686 rs1065852 rs28371725 rs3892097 rs5030655 rs5030867 rs5030865 hCV32407229

Table S2: Threshold used for phenotype assessment.

	PM *(Mean MR ± SD)*	**IM** *(Mean MR ± SD)*	**NM** *(Mean MR ± SD)*	**UM** *(Mean MR ± SD)*
CYP1A2	0.117 ± 0.087	NA	0.33 ± 0.16	0.60 ± 0.18
CYP2B6	0.096 ± 0.034	NA	1.89 ±1.08	8.8 ± 3.9
CYP2C9	0.041 ± 0.011	NA	0.046 ± 0.014	0.090 ± 0.021
CYP2C19	0.18 ± 0.10	NA	0.76 ± 0.46	5.42 ± 2.46
CYP2D6	0.05 ± 0.02	0.46 ± 0.41	2.41 ± 1.79	NA
CYP3A	0.22 ± 0.07	NA	0.57 ± 0.25	3.74 ± 1.50

NA : Not applicable

Table S3: Variant alleles frequencies (%).

Isoforms and variant allele	**Percentage of study population (n)**	**Predicted phenotype**
CYP2B6		
*1/*1	83.3 (25)	NM
*1/*5	16.7 (5)	NM
CYP2C9		
*1/*1	56.7 (17)	NM
*1/*2	23.3 (7)	IM
*1/*3	6.7 (2)	IM
*2/*2	10 (3)	PM
CYP2C19		
*1/*1	50 (15)	NM
*1/*2	13.3 (4)	IM
*1/*4	3.3 (1)	IM
*1/*17	20 (6)	RM

*2/*17	6.7 (2)	IM
*17/*17	3.3 (1)	UM
CYP2D6		
*1/*1	13.3 (4)	NM (AS = 2)
*1/*2	13.3 (4)	NM (AS = 2)
*1/*4	13.3 (4)	IM (AS = 1)
*1/*10	6.7 (2)	NM (AS = 1.25)
*1/*41	3.3 (1)	NM (AS = 1.25)
*2/*2	3.3 (1)	NM (AS = 2)
*2/*4	16.7 (5)	IM (AS = 1)
*2/*41	10 (3)	NM (AS = 1.5)
*4/*4	3.3 (1)	PM (AS = 0)
*4/*12	3.3 (1)	PM (AS = 0)
*6/*12	3.3 (1)	PM (AS = 0)
*10/*41	3.3 (1)	IM (AS = 0.75)

AS = activity score, IM = intermediate metabolizer, NM = normal metabolizer, PM = poor metabolizer, RM = rapid metabolizer, UM = ultra-rapid metabolizer.

Chapter 5: Impact of SARS-CoV-2 Infection (COVID-19) on Cytochromes P450 Activity Assessed by the Geneva Cocktail.

Summary

The beginning of 2020 was marked by the emergence of the serious acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic in Europe and coronavirus disease 2019 (COVID-19). It was a huge health challenge in prevention, creation of diagnostics and medical facilities for immediate detection, treatments of the disease and extensive research for the rapid development of drugs and vaccines to treat and prevent the infection. It quickly becomes apparent that the severe infection with SARS-CoV-2 is endorsed by a hyper-activation of the immune system and the release of a cytokine storm. Indeed, airways are damaged by the aggressive inflammatory response, and the severity of the disease is thus dependent on the host response to the viral infection. Consequently, SARS-CoV-2 infection provided another acute inflammation model. With the same purpose as **research article 2**, the **research article 3** presented in **chapter 5** was conducted to support treatments individualization in the context of this new virus with scarce knowledge. Indeed, **chapter 4** showed that acute inflammation has a clinically significant impact on the main CYPs isoforms (except CYP2D6). Guidelines to manage COVID-19 include drugs that are CYPs substrates and the indication for a dosing regimen adjustment in these patients may thus be potentially needed. Most patients hospitalized for severe COVID-19 have comorbidities with regular treatments. The steady-state of these treatments could be transitorily perturbed by a variability in CYPs expression and activity.

The **research article 3** was also published in *Clinical Pharmacology and Therapeutics* and is a prospective observational study conducted in thirty patients hospitalized with severe COVID-19. Phenotypic activity of the six CYPs assessed with the Geneva cocktail and pro-inflammatory marker levels (CRP, IL-6 and TNF-α) were measured during SARS-CoV-2 infection and three months later. As anticipated, SARS-CoV-2 infection was a good inflammatory model as pro-inflammatory markers levels were significantly higher during infection. The same modulation of CYP activities was found in **research articles 2** and **3**, but results differed in terms of the magnitude of effect. The correlation with several variables such as pro-inflammatory markers levels, BMI, age, gender, diabetes or DDIs were tested.

My contributions to the **research article 3** were the entire management of the clinical study, the recruitment of patients, the collection of samples and data, the assessment of genotype, the analysis of the results and the article's writing.

Research article 3: Impact of SARS-CoV-2 Infection (COVID-19) on Cytochromes P450 Activity Assessed by the Geneva Cocktail.

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Impact of SARS-CoV-2 Infection (COVID-19) on Cytochromes P450 Activity Assessed by the Geneva Cocktail

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Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, is a severe acute respiratory syndrome with an underlying inflammatory state. We have previously demonstrated that acute inflammation modulates cytochromes P450 (CYPs) activity in an isoform-specific manner. We therefore hypothesized that COVID-19 might also impact CYP activity, and thus aimed to evaluate the impact of acute inflammation in the context of SARS-CoV-2 infection on the six main human CYPs activity. This prospective observational study was conducted in 28 patients hospitalized at the Geneva University Hospitals (Switzerland) with a diagnosis of moderate to severe COVID-19. They received the Geneva phenotyping cocktail orally during the first 72 hours of hospitalization and after 3 months. Capillary blood samples were collected 2 hours after cocktail administration to assess the metabolic ratios (MRs) of CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A. C-reactive protein (CRP), interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α) levels were also measured in blood. CYP1A2, CYP2C19, and CYP3A MRs decreased by 52.6% ($P = 0.0001$), 74.7% ($P = 0.0006$), and 22.8% ($P = 0.045$), respectively, in patients with COVID-19. CYP2B6 and CYP2C9 MRs increased by 101.1% ($P = 0.009$) and 55.8% ($P = 0.0006$), respectively. CYP2D6 MR variation did not reach statistical significance ($P = 0.072$). As expected, COVID-19 was a good acute inflammation model as mean serum levels of CRP, IL-6, and TNF-α were significantly ($P < 0.001$) higher during SARS-CoV-2 infection. CYP activity are modulated in an isoform-specific manner by SARS-CoV-2 infection. The pharmacokinetics of CYP substrates, whether used to treat the disease or as the usual treatment of patients, could be therefore clinically impacted.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Genetic, physiological, and environmental factors lead to high interindividual/intraindividual variability in CYP activity. Inflammation can downregulate CYP activity through pretranscriptional and post-transcriptional mechanisms.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ What is the impact of acute inflammation triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection on the activity of the six major human CYP isoforms?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ SARS-CoV-2 infection and subsequent inflammation have an isoform-specific impact on CYP activity, with different magnitudes. Patients with COVID-19 had lower activities of CYP1A2, CYP2C19, and CYP3A. In contrast, CYP2B6 and CYP2C9 activities increased during COVID-19, whereas CYP2D6 activity was unchanged. The isoform-specific impact of SARS-CoV-2 infection on CYP activity was similar to our previous study that evaluated the impact of acute inflammation (hip surgery), but with a different effect size.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Patients with moderate/severe COVID-19 frequently receive CYP substrates to treat the infection and their underlying comorbidities. Awareness of the impact of COVID-19 on drug pharmacokinetics may improve drugs' benefit/risk ratio.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19), so named by the World Health Organization (WHO), emerged in late December 2019. It was identified as being caused by a coronavirus, which is a single-stranded RNA virus, later entitled severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1]

COVID-19 presents as a respiratory infection with a broad spectrum of symptoms.[1] A minority of patients will present a severe to critical disease that could lead to acute respiratory distress syndrome and multiple organ failure.[2] The host inflammatory response has been hypothesized to play an important role in the severity of the disease, with, in severe cases of COVID-19, an uncontrolled response of the immune system with massive release of proinflammatory cytokines.[3] This life-threatening response is characterized by high levels of cytokines and hyperactivation of immune cells, hence the proportionality found between markers of inflammation and disease severity.[4] Indeed, elevated proinflammatory markers, including C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin (IL)-2, IL-4, IL-6, and IL-10 levels, are proportional to COVID-19 severity.[3,5] Moreover, IL-6 and TNF-α were independent and significant predictors of disease severity and death.[6] Similarly, CRP correlated with disease severity and appeared to be a good predictor of adverse outcomes.[7] Studies suggest that CRP levels are an excellent biomarker of the presence and severity of COVID-19, with the advantages that CRP is routinely measured to assess inflammation in patients.[3]

The impact of the release of immunogenic proteins during COVID-19 on CYP activity has not yet been studied, but data on CYP regulation by inflammatory proteins are well described.[8] Indeed, several *in vitro* and animal studies, as well as studies conducted in humans, report that inflammation modulates cytochromes P450 (CYPs) activities.[9,10] Moreover, using a cocktail approach, we have recently demonstrated that inflammation has an isoform-specific impact on CYP and with a different velocity.[11] The underlying mechanisms are thought to be pre-transcriptional and post-transcriptional, with a reduction in messenger RNA levels or inhibition of its translation into protein.[10] Specifically, several case reports of theophylline and clozapine toxicity after the onset of respiratory tract infection are described in the literature.[10,12–14] Authors suggested that the increase of clozapine and theophylline plasma concentrations were linked to CYP1A2 inhibition. Furthermore, pneumonia could inhibit CYP3A according to two case reports studying perampanel and risperidone pharmacokinetic parameters, respectively.[15,16] Similarly, some authors have started to investigate the impact of COVID-19 on CYP substrates, and available results were reviewed.[8] The plasma concentrations of some CYP3A substrates (lopinavir, darunavir, and direct oral anticoagulants) were indeed shown to be significantly higher in patients with COVID-19.[17–20] Lopinavir concentrations were also associated with CRP and IL-6 levels as they decreased after tocilizumab administration in patients with COVID-19.[18,21] Finally, clozapine toxicity symptoms and increased clozapine level were reported during COVID-19.[22] These findings warrant further investigation, as patients with severe COVID-19 often have several comorbidities and treatments, and some drugs administered to patients with COVID-19 are CYP substrates.[23,24] Thus, the probability that patients with COVID-19 received CYP substrates is high and these isoenzymes are known to have interindividual and intraindividual variability over a period of time, which are the consequences of the interplay between genetic, environmental, and physiological factors.[10]

We therefore sought to evaluate the effects of moderate to severe COVID-19 as a model of acute inflammation on the activity of the six major CYPs in patients hospitalized with SARS-CoV-2 infection, using a phenotyping cocktail approach. To our knowledge, this is the first time that the impact of COVID-19 has been assessed simultaneously on the six main human CYPs.

METHODS

Study protocol

This study assessed the impact of moderate to severe COVID-19 on the activities of the six main human CYPs, namely CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A, through a prospective open-label observational study. The regional research ethics committee of the canton of Geneva (CCER) approved the amendment to the study protocol (No. 2016-02232), and the study was registered with the US National Institutes of Health Clinical Trials Registry (NCT03262051). All patients gave written informed consent before the start of any study procedure. The principles of the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice were followed.

Study population

Participants were recruited within the first 72 hours of hospitalization at the Geneva University Hospitals for COVID-19 over a period from October 30 to December 12, 2020. Inclusion and exclusion criteria are described in **Table S1**. World Health Organization (WHO) criteria were used to assess the severity of COVID-19.[25] Comedications were systematically run through the Lexi-Interact drug interaction checker and the Geneva table of CYPs to identify CYP inhibitors and inducers.[26,27] Patients receiving dexamethasone were not excluded because it is currently a standard of care for the management of hospitalized patients with COVID-19.[28] To limit the inducing effect of dexamethasone on CYP3A activity, only patients who received dexamethasone 5 mg once daily up to two times were included.

The primary objective was to measure the variation in activity of the six major human CYPs during and 3 months after (defined as baseline) SARS-CoV-2 infection.

Genotyping of CYP2B6, CYP2C9, CYP2C19, and CYP2D6

The method used to genotype CYP2B6, CYP2C9, CYP2C19, and CYP2D6 has already been described in detail in the literature.[29] Genetic profile information from genotyping (single-nucleotide variants) and copy number assay were translated using the same software as in our previous study conducted in patients who underwent elective hip surgery.[11]

Phenotyping

Phenotype assessment technique has been previously described.[11] CYP activity and subsequent phenotypic classification were based on metabolic ratios (MRs), defined as the concentration of the metabolite divided by the concentration of substrate. These concentrations were assessed by a validated method using liquid chromatography–tandem mass spectrometry quantification.[30–32] Based on their MRs for each CYP, patients were classified as poor metabolizers (PMs), normal metabolizers (NMs), and ultra-rapid metabolizers (UMs), as well as intermediate metabolizers for CYP2D6. Threshold values were the same as those already detailed in our previous cohort study.[11]

The MRs of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A were measured twice, i.e., during the first 72 hours of the patient's hospitalization and 3 months after. To assess the phenotype of each CYP of interest, probe substrates contained in the Geneva cocktail (caffeine 50 mg, CYP1A2; bupropion 20 mg, CYP2B6; flurbiprofen 10 mg, CYP2C9; omeprazole 10 mg, CYP2C19; dextromethorphan 10 mg, CYP2D6; midazolam 1 mg, CYP3A; fexofenadine 25 mg, P-glycoprotein) were orally administered and capillary blood samples were collected 2 hours later from a fasting patient, with dried blood spots using a previously validated sampling method.[30] Phenotypic P-glycoprotein (P-gp) activity was not assessed because it requires an area under the curve (AUC) of fexofenadine blood concentration (two additional capillary blood samples required 3 and 6 hours later) and this was deemed inappropriate in the context of hospital overload during the second wave of SARS-CoV-2 infection. Dried blood spots were then stored at −20°C in a sealable plastic bag until analysis, as previously described.[33] No mutual drug–drug interactions were observed in the Geneva cocktail.[34] CYP2D6 was not modulated by bupropion because of the extremely low doses and time intervals used.[34]

Inflammatory markers levels

Whole blood samples with lithium heparin and without additive were collected twice in the early morning, namely during the first 72 hours of patients' hospitalization and 3 months later, respectively, to assess CRP, IL-6, and TNF-α levels. The analysis methodology is described in detail in our previous study.[11]

Data and statistical analysis

A sample size of 16 subjects was required to detect > 30% reversal of CYP3A activity with 80% power and an α value of 5%. In terms of correlation of CYP function with IL-6 (and other proinflammatory markers), a sample size of 24 subjects was required to consider a coefficient of 0.55 as significant, with 80% power and an α value of 5%. The sample size of 24 subjects allows detection of a > 22% difference in CYP MRs between pairs, assuming that the standard deviation (SD) of the differences is 36% (literature estimate of MR standard deviation for CYP3A). To prevent loss to follow-up, a sample size of 30 subjects was targeted. A P value < 0.05 was considered statistically significant, and IBM SPPSS Statistics software version 25 (Chicago, IL) was used to perform all statistical analyses. Continuous variables were described as means ± SD and a paired t-test was used to determine the percentage difference in MRs and levels of inflammatory markers before and after COVID-19. After testing for normality by the Kolmogorov-Smirnov test and finding that the normality assumption was generally not met, a nonparametric Spearman correlation test was applied. Spearman correlations were assessed between different variables such as variation (delta) in inflammatory markers levels and CYP MRs, body mass index (BMI), and age (continuous variable), and a t-test was applied between variation (delta) of CYP MRs and sex, dexamethasone use, COVID-19 severity classification (severe vs. moderate), or diabetic status (binary variables). Continuous variables were standardized. A multiple linear regression model was built to evaluate the inflammatory markers influencing the variation (delta) in CYP activity (dependent variables) observed during and after COVID-19 by controlling the other predictors put in the model. The independence between all the variables was verified using a collinearity test.

RESULTS

Demographic

Thirty subjects were included for the first part of the study, but two withdrew their consent for the second part of the study (3 months later) and were thus excluded. The summary of patients' demographics and clinical characteristics is presented in **Table S2**. Hospitalization and inclusion after symptoms onset were based on 27 patients, as one patient was hospitalized on the day of incidental discovery of infection.

Proinflammatory markers

The effect of SARS-CoV-2 infection on inflammatory markers (CRP, IL-6, and TNF-α) serum levels are shown in **Figure 1** and **Table 1**.

CYP activity during and after SARS-CoV-2 infection

Table 2 shows the activities of the 6 CYPs of interest during (acute inflammation) and 3 months after (baseline levels) SARS-CoV-2 infection. CYP1A2, CYP2C19, and CYP3A MRs decreased by 52.6% (P = 0.0001), 74.7% (P = 0.0006), and 22.8% (P = 0.045), respectively, during SARS-CoV-2 infection. Inversely, CYP2B6 and CYP2C9 MRs increased by 101.1% (P = 0.009) and 55.8% (P = 0.0006), respectively, while the 35.2% increase of CYP2D6 MRs did not reach statistical significance (P = 0.072).

Phenoconversion

Table S3 shows the patients' genotype with allele frequencies and predicted phenotype from genotype for each CYP. The predicted phenotype matched the measured phenotype 3 months after COVID-19 in 82.1%, 64.3%, and 75.0% of patients for CYP2B6, 2C19, and 2D6, respectively. For 82.1% of patients, the predicted phenotype for CYP2C9 did not reflect the measured phenotype 3 months after SARS-CoV-2 infection. Almost all (78.6%) of them had an accelerated CYP2C9 measured phenotype compared with the predicted phenotype. For CYP2C19, 17.9% of patients had a decreased measured phenotype 3 months after COVID-19 compared with the predicted phenotype.

A phenotypic switch from NM to PM or from UM to NM was observed in 71%, 46%, and 43% of subjects for CYP1A2, CYP2C19, and CYP3A, respectively, during COVID-19 (**Figure 2a–c**). Fifty-four percent of subjects were CYP2C19 PMs 3 months after COVID-19 (**Figure 2b**). Phenoconversion from PM to NM or from NM to UM was observed in 36% and 29% of subjects for CYP2B6 and CYP2C9, respectively (**Figure 2d,e**). Twenty out of the 28 included patients had no CYP2C9 phenoconversion, but 19 of them were CYP2C9 UMs 3 months after COVID-19 (**Figure 2e**). Concerning CYP2D6, no change of phenotypic category was observed in 79% of subjects (**Figure 2f**).

Variables that influenced the change in CYP activity

Table 3 shows Spearman correlations performed on the variation of the MRs of CYP isoform during and 3 months after COVID-19, and different factors, such as variation of proinflammatory markers, BMI, sex, age, COVID-19 severity, diabetic status, or dexamethasone intake. No correction for multiple testing was performed. An increased level of CRP was associated with a more marked inhibition of CYP3A, and the older the patients, the more CYP2C19 and CYP2B6 were inhibited (significant negative association), and CYP2C19 activity was higher in women (significant positive association).

A multiple linear regression model was built to assess factors associated with variation of CYP activity while controlling the other predictors put in the model, such as variation of proinflammatory

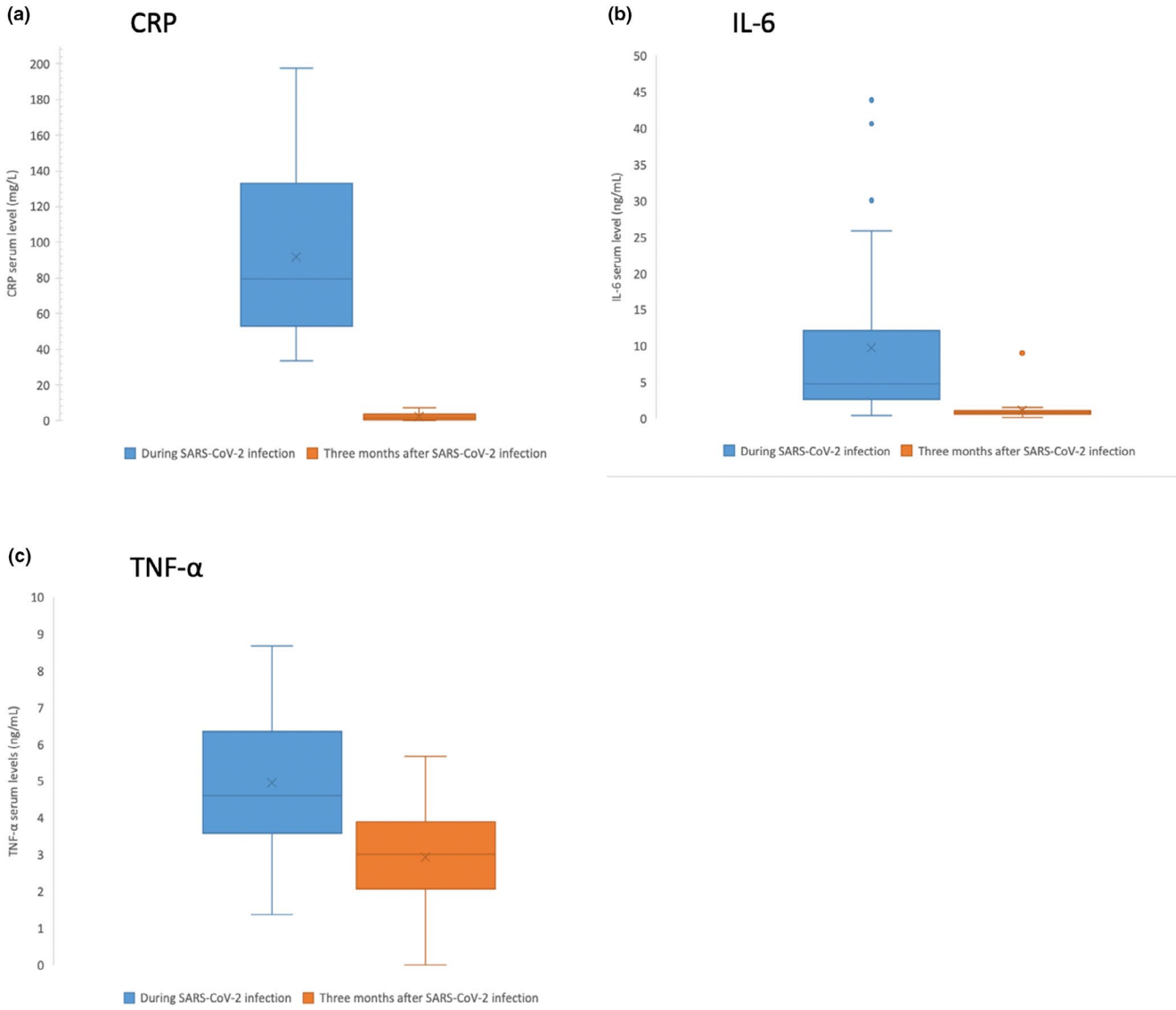

Figure 1 Serum levels of the three inflammatory markers (**a**) CRP, (**b**) IL-6, and (**c**) TNF-α during and 3 months after SARS-CoV-2 infection (n = 28). The boundary of the box closest to zero indicates the 25th percentile, the black line within the box marks the median, the cross within the box marks the mean, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whiskers indicate outliers. CRP, C-reactive protein; IL-6, interleukin 6; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF- α, tumor necrosis factor-α. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1 Mean MRs ± SD of the three inflammatory markers

Inflammatory markers	Serum levels units	During COVID-19	After COVID-19	*P* value
CRP	mg/L	91.7 ± 44.6	2.4 ± 1.9	4.02×10^{-11}
IL-6	ng/mL	9.72 ± 11.77	1.14 ± 1.58	7.86×10^{-4}
TNF-α	ng/mL	4.95 ± 1.96	2.94 ± 1.16	8.20×10^{-7}

Mean MRs ± SD of the three inflammatory markers measured during and 3 months after SARS-CoV-2 infection ($n = 28$) ($P < 0.05$ is significant).
COVID-19, coronavirus disease 2019; CRP, C-reactive protein; IL-6, interleukin 6; MRs, metabolic ratios; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNFα, tumor necrosis factor-α.

markers, BMI, sex, age, COVID-19 severity, diabetic status, or dexamethasone intake. Independence was tested by a collinearity test (variation inflation factor), and all the covariables were independent of each other. However, the focus was on variation in proinflammatory markers in relation to variation in CYP activity (**Table 4**).

The model was a significant predictor of variations in CYP1A2 and CYP2D6 MRs but not for CYP2B6, CYP2C9, CYP2C19, and CYP3A4, as shown in **Table S4**. The same associations between variation in CRP, IL-6, and TNF-α levels and CYP MRs were not found in the multiple linear regression model compared

Table 2 Mean MRs ± SD of the six CYP isoforms

Isoforms	MRs parameters ((Mean) ± SD)	During SARS-CoV-2 infection	3 months after SARS-CoV-2 infection	*P* value
CYP1A2	(paraxantine) / (caffeine)	0.199 ± 0.081	0.420 ± 0.258	0.0001
CYP2C19	(OH-omeprazole) / (omeprazole)	0.148 ± 0.129	0.586 ± 0.671	0.0006
CYP3A	(OH-midazolam) / (midazolam)	0.428 ± 0.289	0.550 ± 0.240	0.045
CYP2B6	(OH-bupropion) / (bupropion)	2.263 ± 2.502	1.324 ± 0.844	0.009
CYP2C9	(OH-flurbiprofen) / (flurbiprofen)	0.120 ± 0.062	0.077 ± 0.031	0.0006
CYP2D6	(dextrophan) / (dextromethorphan)	3.010 ± 2.381	2.226 ± 2.078	0.072

Mean MRs ± SD of the six CYP isoforms during and 3 months after SARS-CoV-2 infection (n = 28) ($P < 0.05$ is significant).
CYP, cytochrome P450; MRs, metabolic ratios; OH, hydroxy; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

with Spearman correlations. Indeed, variation in CRP levels was associated with variation in CYP3A MRs, IL-6 levels with CYP1A2 and CYP2C9, and TNF-α levels with CYP2D6. This could be explained by the fact that each proinflammatory marker was controlled by the other two, and the release of CRP and TNF-α is initiated by IL-6. The variation in TNF-α level was removed because the difference was small between the COVID-19 stage and 3 months later, and this variation was almost within the expected ranges of variability. The new model thus significantly predicted the variation in CYP2C9 and CYP3A activity, as shown in **Table S4**. These coefficients of variation and *P* value associated with the change in serum CRP and IL-6 levels were not modified in this model compared with the first model integrating TNF-α change.

Therefore, the change in activity of some CYPs observed during SARS-CoV-2 infection correlated with several variables, such as variation in CRP levels (CYP3A), IL-6 levels (CYP1A2 and CYP2C9), and TNF-α levels (CYP2D6), sex (CYP2C19), and age (CYP2C19 and CYP2B6). BMI, diabetic status, dexamethasone intake, and COVID-19 severity were not correlated with CYP variations observed during SARS-CoV-2 infection.

Smoking status and initiation of CYP modulator treatments between the beginning and end of the study were not taken into account because they involved only one and three patients, respectively. Moreover, only CYP3A and CYP2C19 inhibitors were initiated and these CYPs were already inhibited during SARS-CoV-2 infection; thus, the only consequence would have been an offset of the inhibitory effect of inflammation on CYP3A and CYP2C19 activities during SARS-CoV-2 infection, which was not observed.

DISCUSSION

We have demonstrated that SARS-CoV-2 infection has an isoform-specific impact on the activity of the six main human CYPs, with different effect and magnitude. To our knowledge, this is the first time that a cocktail approach was used to study CYP activity in COVID-19.

To date, only five studies and one case report have reported the impact of SARS-CoV-2 infection on CYP substrates, but not on probe drugs.[17–22] Indeed, one case report described the onset of symptoms of clozapine toxicity associated to a clozapine level that increased after COVID-19.[22] In addition, lopinavir/ritonavir as well as darunavir, all of which are CYP3A substrates, have been used as a treatment for SARS-CoV-2 infection. Their trough concentrations were significantly higher and their clearances lower in patients with COVID-19 compared with patients with HIV.[17,18,20] Lopinavir plasma concentrations were associated with CRP levels in patients with COVID-19 and were significantly lower when tocilizumab was administered beforehand.[18,21] Finally, direct oral anticoagulants are also CYP3A substrates and an alarming increase in their plasma levels was observed, as compared with prehospitalization levels.[19] However, a possible role of concomitant drugs or disease-related organ dysfunction cannot be excluded.[19]

The isoform-specific impact of SARS-CoV-2 infection on CYP activity was similar to our previous study that evaluated the impact of an another acute inflammation model (hip surgery).[11] However, the effect size was higher for CYP2C19 and lower for CYP3A, CYP2B6, and CYP2C9. It was similar for CYP1A2 and CYP2D6.

CYP2C19 was the most downregulated CYP, with a decrease by 75% during SARS-CoV-2 infection, and the decreased activity was inversely correlated with IL-6 and CRP levels. In our previous cohort study, CYP2C19 activity decreased by 57% and was inversely correlated with CRP levels.[11] This is in accordance with previous publications that demonstrated a decrease of CYP2C19 activity during an inflammatory condition, and negative correlations with IL-6 and TNF-α.[35,36] Moreover, the ratio of clopidogrel active metabolite (bioactivated by CYP2C19) to clopidogrel has been shown to be 48-fold higher in healthy subjects than in critically ill patients, and platelet aggregation was significantly higher in patients with elevated CRP levels.[37,38]

We could not demonstrate correlation between the variations of CYP2C19 MR and any of the proinflammatory markers. Difference in the kinetics of these variables might explain the absence of correlation, due to an expected time lag between elevation of proinflammatory markers and CYP downregulation. Furthermore, proinflammatory markers were measured during the first 72 hours of hospitalization in patients with COVID-19 and so a discordance in proinflammatory marker levels could exist among our included patients because they were not hospitalized at exactly the same time after disease onset, or they were not included exactly

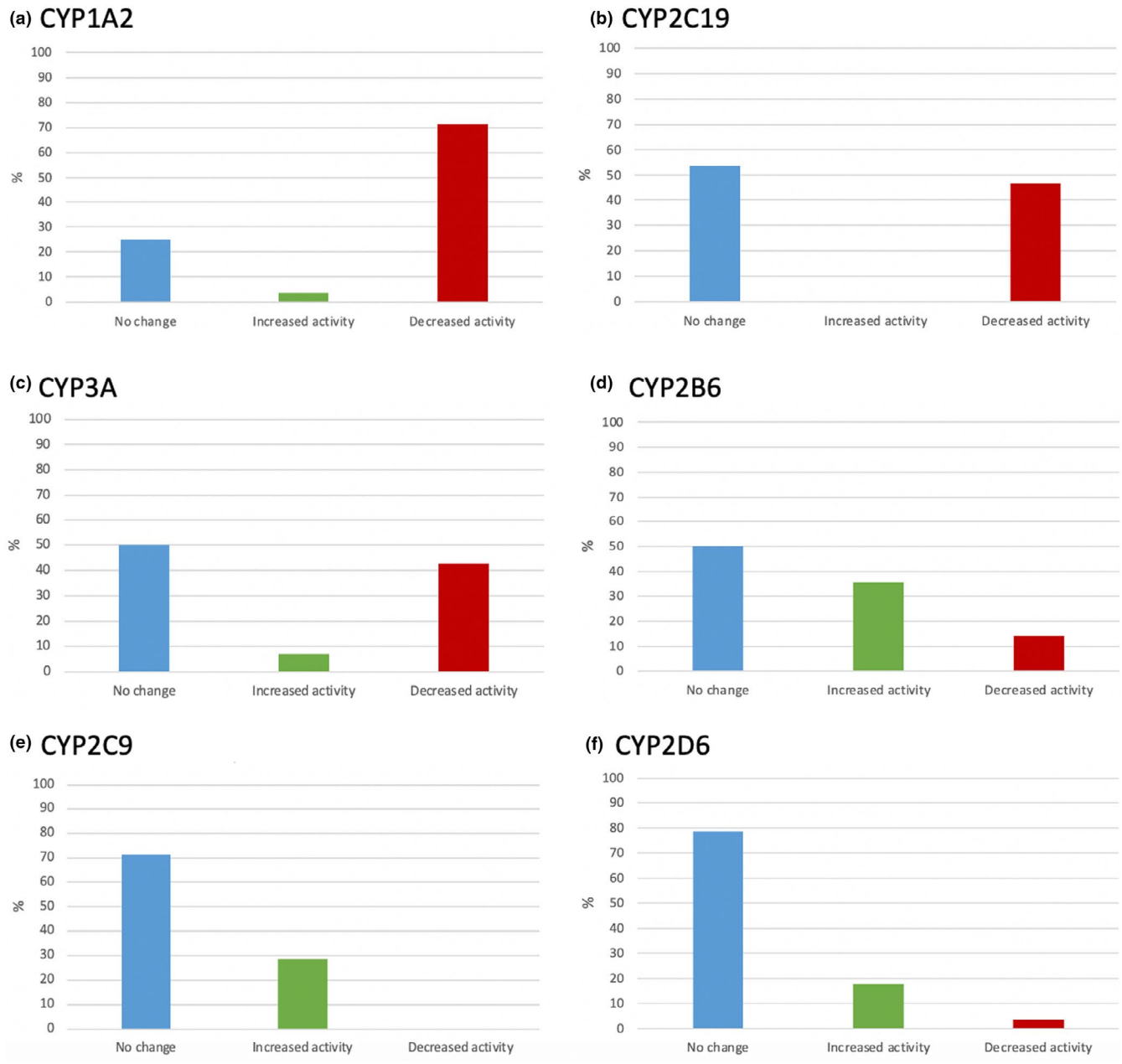

Figure 2 Percentage of patients (n = 28) with CYP phenotypic switch between 3 months after (baseline) and during SARS-CoV-2 infection: (**a**) CYP1A2, (**b**) CYP2C19, (**c**) CYP3A, (**d**) CYP2B6, (**e**) CYP2C9, and (**f**) CYP2D6. CYP, cytochrome P450; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. [Colour figure can be viewed at wileyonlinelibrary.com]

at the same time after the beginning of their hospitalization. It is particularly important to note that phenoconversion was observed in 100% of patients who were not PMs at baseline. Indeed, the phenoconversion observed in slightly less than half of the subjects, as shown in **Figure 2b**, can be explained by the fact that half of the individuals carried alleles associated with decreased CYP2C19 activity (**Table S3**). Moreover, out of the three NM patients predicted on the basis of genotype who had a PM phenotype 3 months after SARS-CoV-2 infection, one was started on esomeprazole, a well-known CYP2C19 inhibitor. We cannot exclude that the other two took CYP2C19 inhibitors without informing us.

We found that CYP1A2 was the second-most downregulated CYP with a decrease of 53% during SARS-CoV-2 infection, with inverse correlation with IL-6 and CRP levels. The same magnitude and correlations were found for CYP1A2 in hip surgery patients.[11] These results are in agreement with previous published studies, since many case reports have described increased clozapine and theophylline toxicity or plasma concentrations during inflammatory conditions, such as infection or elevated levels of CRP.[10,12–14] IL-6 but not TNF-α levels have been inversely correlated with CYP1A2 activity in 16 patients with congestive heart failure.[35] Recently, a case report of clozapine toxicity with increased level during

Table 3 Correlation between change in CYPs MRs and change in serum pro-inflammatory markers levels

	Δ CYP1A2	Δ CYPC19	Δ CYP3A	Δ CYP2B6	Δ CYP2C9	Δ CYP2D6
Δ CRP	*r* = −0.305 (*P* = 0.115)	*r* = −0.090 (*P* = 0.648)	*r* = −0.516 (*P* = 0.005)	*r* = −0.076 (*P* = 0.700)	*r* = −0.183 (*P* = 0.352)	*r* = −0.084 (*P* = 0.672)
Δ IL-6	*r* = −0.068 (*P* = 0.730)	*r* = 0.178 (*P* = 0.364)	*r* = 0.063 (*P* = 0.751)	*r* = −0.117 (*P* = 0.554)	*r* = 0.225 (*P* = 0.250)	*r* = 0.092 (*P* = 0.643)
Δ TNF-α	*r* = 0.005 (*P* = 0.980)	*r* = −0.139 (*P* = 0.480)	*r* = −0.137 (*P* = 0.486)	*r* = −0.143 (*P* = 0.467)	*r* = 0.093 (*P* = 0.638)	*r* = 0.449 (*P* = 0.017)
Sex	*t* = 1.683 (*P* = 0.104)	*t* = 2.940 (*P* = 0.007)	*t* = −0.920 (*P* = 0.366)	*t* = 1.211 (*P* = 0.237)	*t* = −1.060 (*P* = 0.299)	*t* = −0.119 (*P* = 0.906)
Age	*r* = −0.109 (*P* = 0.581)	*r* = −0.487 (*P* = 0.009)	*r* = −0.037 (*P* = 0.852)	*r* = −0.493 (*P* = 0.008)	*r* = −0.018 (*P* = 0.928)	*r* = 0.039 (*P* = 0.842)
BMI	*r* = 0.060 (*P* = 0.760)	*r* = −0.192 (*P* = 0.327)	*r* = −0.141 (*P* = 0.473)	*r* = 0.201 (*P* = 0.306)	*r* = −0.067 (*P* = 0.736)	*r* = −0.001 (*P* = 0.997)
COVID-19 severity (moderate vs. severe)	*t* = −0.716 (*P* = 0.480)	*t* = 0.460 (*P* = 0.649)	*t* = 0.281 (*P* = 0.781)	*t* = 1.819 (*P* = 0.080)	*t* = −0.811 (*P* = 0.475)	*t* = −1.171 (*P* = 0.252)
Diabetic status	*t* = 1.006 (*P* = 0.324)	*t* = 8.858 (*P* = 0.399)	*t* = −0.375 (*P* = 0.710)	*t* = 2.112 (*P* = 0.086)	*t* = −0.261 (*P* = 0.796)	*t* = 0.167 (*P* = 0.869)
dexamethasone intake	NA	NA	*t* = −0.252 (*P* = 0.803)	NA	NA	NA

Correlation (Spearman) between change in MRs (delta) of the six CYP isoforms and change (delta) is serum IL-6, TNF-a and CRP levels during and 3 months after SARS-CoV-2 infection in the 28 subjects ($P < 0.05$ is significant). BMI, body mass index; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; CYP, cytochrome P450; IL-6, interleukin 6; MRs, metabolic ratios; NA, not applicable; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNFα, tumor necrosis factor-α.

Table 4 Linear regression model of the difference in CYPs MRs

	Δ CYP1A2	Δ CYPC19	Δ CYP3A	Δ CYP2B6	Δ CYP2C9	Δ CYP2D6
Δ CRP	−0.342 (SE = 0.174) *P* = 0.060	−0.242 (SE = 0.191) *P* = 0.218	−0.468 (SE = 0.182) *P* = 0.017	−0.031 (SE = 0.200) *P* = 0.878	−0.151 (SE = 0.181) *P* = 0.411	−0.302 (SE = 0.170) *P* = 0.089
Δ IL-6	−0.439 (SE = 0.178) *P* = 0.021	0.229 (SE = 0.196) *P* = 0.255	0.084 (SE = 0.186) *P* = 0.654	−0.068 (SE = 0.205) *P* = 0.744	0.443 (SE = 0.185) *P* = 0.025	0.074 (SE = 0.175) *P* = 0.677
Δ TNF-α	0.060 (SE = 0.180) *P* = 0.742	−0.204 (SE = 0.198) *P* = 0.313	0.008 (SE = 0.188) *P* = 0.967	−0.210 (SE = 0.207) *P* = 0.322	0.057 (SE = 0.187) *P* = 0.764	0.496 (SE = 0.176) *P* = 0.010

Standardized variables in the linear regression model and association with the difference in metabolic activits of the six CYP isoforms during and 3 months after SARS-CoV-2 infection in the 28 subjects ($P < 0.05$ is significant). CRP, C-reactive protein; CYP, cytochrome P450; IL-6, interleukin 6; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNFα, tumor necrosis factor-α.

SARS-CoV-2 infection was described.[22] The impact of inflammation appears to be linked to disease severity, as metabolic status of caffeine did not change in HIV-infected asymptomatic patients but decreased in patients with AIDS (with acute illnesses).[39] We found a phenotypic switch in 71% of included patients.

The decrease in CYP3A activity by 23% during SARS-CoV-2 infection was of smaller magnitude than in hip surgery patients (60% decrease).[11] This may be due in part to the use of dexamethasone, which is known to be a weak inducer of CYP3A,[40] even if no correlation was found. Moreover, one patient started amlodipine between the end of his hospitalization and 3 months later (baseline). This may explain reduced activity at baseline and an apparently reduced downregulation of CYP3A activity by inflammation, as amlodipine is considered a weak CYP3A4 inhibitor.[40] Furthermore, in an acute inflammation surgery model, we previously showed that the maximal decrease of CYP3A activity occurred after 3 days, and therefore maximal inhibition of CYP3A might not have been reached at the time of measurement.[11] Still, 43% of patients experienced a phenoconversion during SARS-CoV-2 infection. We found an inverse correlation with CRP levels, which is in accordance with a previous study in proportion to disease severity.[41] Lopinavir trough concentrations also significantly increased and were positively correlated with CRP levels in patients with COVID-19.[18,21]

We showed that CYP2B6 activity increased by 100% during SARS-CoV-2 infection with significant and positive correlations with CRP levels, although not significant when the variations of MR and inflammatory markers were used in the model. These results are in accordance with those found in surgery patients.[11] However, phenoconversion was observed in 36% of patients only. CYP2B is the most inducible CYP isoform by phenobarbital-type compounds in most mammalian species.[42,43] The glucocorticoid

receptor may be acting as a regulation factor as a consequence of cortisol secretion in patients with COVID-19 and stress may thus explain the observed CYP2B6 induction.[42,43] A cohort study indeed showed that median cortisol concentration in patients with COVID-19 was significantly higher than controls ($P < 0.0001$) and that the patients with COVID-19 had a marked acute cortisol stress response.[44] Therefore, cortisol might be a marker of disease severity.[44]

CYP2C9 activity increased by 56% in SARS-CoV-2 infection, while it increased by 79% after surgery.[11] This could be of clinical relevance since phenoconversion was demonstrated in 89% of patients who were not UMs at baseline. Surprisingly 19 out of 28 patients in the cohort were UMs 3 months after SARS-CoV-2 infection while no genetic variant is currently known to increase CYP2C9 activity and there was no CYP2C9 inducer in the comedications.[45] The persistent induced activity of CYP2C9 could be explained either by an unidentified environmental factor or by the existence of as yet undescribed genetic variants. Moreover, the validated cutoff values of the Geneva cocktail for CYP2C9 are based on a study in which volunteers were simultaneously administered rifampin and fluconazole, a CYP2C9 inducer and a CYP2C9 inhibitor, respectively, which are not specific to CYP2C9. Indeed, a very low correlation (17.9%) between the predicted phenotype and the measured phenotype at baseline level was found in this cohort. It is gradually recognized that SARS-CoV-2 can induce long-term complications after recovery from the acute effects of infection, even if these long-term health consequences remain largely unclear.[2,46] According to the National Institute for Health and Care Excellence (NICE), long COVID-19 is a range of symptoms that can last weeks or months after first being infected with the virus.[47] In the United Kingdom, around one in five people who tested positive for COVID-19 had symptoms that lasted for 5 weeks or longer, and one in ten people had symptoms that lasted for 12 weeks or longer.[47] One recent study showed that only 12.6% of patients were completely free of any COVID-19 symptoms after 60 days and that 55% still had three or more symptoms.[48] Another study with a longer follow-up period showed that 24.1% of patients still had at least one symptom after 90 days, this figure reaching 40.6% in those with more severe initial acute disease.[46] We hypothesize that CYP2C9 activity levels measured 3 months after infection could be associated with long COVID-19 metabolic disturbances, yet to be identified. Indeed, ~ 30% of our included patients still described long-term effects of COVID-19 at 3 months. It would thus be of interest to reassess CYP activity in our cohort of patients with COVID-19 with a much longer delay to further support this hypothesis. Indeed, it is estimated that recovery of CYP activity after discontinuation of inducers can be achieved in 14 days, which is longer than after discontinuation of mechanism-based (10 days) or competitive inhibitors (which depend on their elimination half-life).[49]

Finally, COVID-19 had no significant impact on CYP2D6 activity, as already observed in surgery-induced acute inflammation.[11] A recent cohort study did not find any correlation between CRP and hydroxychloroquine plasma concentration in patients with COVID-19, treated or not with tocilizumab.[21] CYP2D6 activity was not influenced by diabetic status either.[50] However, conflicting results have been published in patients infected with HIV.[51,52] This observation could be explained by the fact that CYP2D6 has a high intraindividual variability, and dextromethorphan MR can vary up to 50% within healthy subjects.[53] The significant Spearman correlation and β coefficient found between the change in TNF-α level and the change in CYP2D6 MR between SARS-CoV-2 infection and situation 3 months later should be taken with caution. Indeed, the change in TNF-α level was small and within the range of variability.

A longitudinal study in patients with COVID-19 previously showed that TNF-α levels peaked 3 to 6 days after disease onset and no difference in their levels was observed between the mild and severe groups.[2] IL-6 reached its serum peak between days 7 and 9 after disease onset in patients with mild COVID-19, whereas the reduction in serum IL-6 levels in severe patients began 16 days after disease onset. In another longitudinal analysis of hospitalized patients with COVID-19, median TNF-α and IL-6 levels in non-critically ill patients were 7.3 pg/mL and 5.0 pg/mL, respectively, during the first 3 days of hospitalization.[54] These figures are comparable to the mean levels found in our cohort, where the mean concentrations were 9.72 and 4.95 ng/mL, respectively. In a retrospective study, mean CRP levels at admission were 16.76, 54.15, and 105.00 mg/L in the moderate, severe, and critical groups, respectively.[55] These results are comparable to the mean CRP level of 91.7 mg/L found in the first 72 hours of admission in our study.

We thus have demonstrated that COVID-19 has an impact on CYP activity in an isoform-specific manner (inhibition or induction, and magnitude). The magnitude of the effects found on CYP1A2, CYP2C19, CYP3A, CYP2B6, and CYP2C9 activities might be of clinical relevance, in particular in polymorbid and polymedicated patients with COVID-19.

Our study has some limitations. First, the sample size was relatively small and confirmation of our multiple linear regression model findings in an additional and/or broader sample is needed, allowing for possible adjustment with other covariables. In addition, a correlation between COVID-19 and variation in CYPs' activity was found, but further investigations are needed to corroborate it. In particular, the patients included had different health status, such as hypertension, diabetes, dyslipidemia, or none. Finally, the duration of follow-up was of only 3 months and there is no guarantee that CYP activity in included patients had returned to their initial levels, in light of considerations about the potential long-term effects of COVID-19. A study with a longer follow-up time may provide answers and should include the statement of symptoms of long COVID-19.

To conclude, our results suggest that SARS-CoV-2 infection and the resulting acute inflammation have a large impact on the activity of six key CYPs in an isoform-specific manner. These effects could be prolonged for certain isoforms. Our findings may help manage relevant drug efficacy and safety issues in the context of COVID-19 through the impact on the PK of drugs that are substrates of these major drug-metabolizing enzymes, whether used to treat acute disease or as routine patient therapy.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

C.L., J.T., and C.F.S. wrote the manuscript. C.L., J.T., Y.G., F.C., V.R., Y.D., J.A.D., J.-L.R., and C.F.S. designed the research. C.L. performed research. C.L., J.T., F.C., and C.F.S. analyzed the data. C.L., Y.G., and Y.D. contributed new reagents/analytical tools.

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Table S1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Older than 18 years	Pregnancy
Positive SARS-CoV-2 nasopharyngeal smear	Breastfeeding
CRP level higher than 30 mg/L	Allergy to any of the components of the Geneva cocktail (caffeine, flurbiprofen, omeprazole, bupropion, dextrometorphan, fexofenadine and midazolam)
Moderate to severe COVID-19 according to World Health Organization (WHO) criteria	Hepatic impairment (defined as transaminases, bilirubin, gamma glutamyl transferase > 2x the upper limit of normal)
	Renal impairment (defined as serum creatinine concentration > 1.5x upper limit of normal)
	Severe heart failure
	Severe edema or ascites
	Active cancer
	Uncontrolled infection other than COVID-19
	HIV infection
	Inflammatory arthritis
	Concomitant treatment with CYP inhibitors or inducers except dexamethasone

Table S2: Summary of demographics and clinical characteristics of patients who completed the entire study (n = 28)

Parameters	Mean ± SD or Number (%)
Age	61 ± 14 years
Age < 65 years	17 (60.8%)
65 years < Age < 80 years	9 (32.1%)
Age > 80 years	2 (7.1%)
Body Mass Index (BMI)	29 ± 4 kg/m^2
BMI < 25	1 (3.6%)
25 < BMI < 30	19 (67.8%)
30 < BMI < 35	4 (14.3%)
35 < BMI < 40	3 (10.7%)
BMI > 40	1 (3.6%)
Female	5 (17.9%)
Male	23 (82.1%)
Caucasian	21 (75.0%)
Asian	5 (17.9%)
African	2 (7.1%)
Severe COVID-19	24 (85.7%)
Moderate COVID-19	4 (14.3%)
Days between onset of symptoms and hospitalization (n=27)	7 ± 2 days (Ranging from 4-12 days)
Days between onset of symptoms and inclusion (n=27)	9 ± 2 days (Ranging from 5-14 days)
Long COVID-19	9 (32.1%)
Type II diabetes	6 (21.4%)
Smokers	1 (3.6%)
Caffeine consumers	28 (100%)
No dose of dexamethasone before onset of study	8 (28.6%)
1 dose of dexamethasone before onset of study	5 (17.9%)
2 doses of dexamethasone before onset of study	15 (53.6%)
CYP3A4 inhibitor at three months	1 (amlodipine)
CYP2C19 inhibitor at three months	2 (esomeprazole and omeprazole)

Table S3: Variant alleles frequencies (%) (n = 30)

Isoforms and variant allele	Percentage of study population (n)	Predicted phenotype
CYP2B6		
*1/*1	80.0% (24[#])	NM
*1/*5	13.3% (4[#])	NM
*1/*22	3.3% (1)	RM
*5/22	3.3% (1)	RM
CYP2C9		
*1/*1	70.0% (21)	NM
*1/*2	16.7% (5[#])	IM
*1/*3	10.0% (3)	IM
*3/*3	3.3% (1[#])	PM
CYP2C19		
*1/*1	26.7% (8[##])	NM
*1/*2	33.3% (10)	IM
*1/*6	3.3% (1)	IM
*1/*17	23.3% (7)	RM
*2/*17	10.0% (3)	IM
UND	3.3% (1)	NA
CYP2D6		
*1/*1 or *1x2/*5	16.7% (5)	NM (AS=2)
*1/*1x2	3.3% (1)	UM (AS=3)
*1/*2	13.3% (4)	NM (AS=2)
*1/*4	10.0% (3)	IM (AS=1)
*1/*14	3.3% (1[#])	NM (AS=1.5)
*1/*41x2 or *1x2/*41	3.3% (1)	NM (AS=2) or UM (AS=2.5)
*1/*4x2 or *1x2/*4	3.3% (1)	IM (AS=1) or NM (AS=2)
*1/*9	3.3% (1)	NM (AS=1.5)
*2/*10	3.3% (1)	NM (AS=1.25)
*2/*2 or *2x2/*5	3.3% (1)	NM (AS=2)
*2/*2x2	3.3% (1)	UM (AS=3)
*2/*4	3.3% (1)	IM (AS=1)
*2/*41	6.7% (2[#])	NM (AS=1.5)
*2/*6	3.3% (1)	IM (AS=1)
*4/*4 or *4x2/*5	6.7% (2)	PM (AS=0)

*4/*41	3.3% (1)	IM (AS=0.5)
*5/*41	3.3% (1)	IM (AS=0.5)
*5/*41x2 or *41/*41	3.3% (1)	IM (AS=1)
*6/*10	3.3% (1)	IM (AS=0.25)

AS = activity score, IM = intermediate metabolizer, NM = normal metabolizer, PM = poor metabolizer, RM = rapid metabolizer, UM = ultra-rapid metabolizer, NA = Not available for technical issues , # = genotype of the two subjects withdrawn from the study

Table S4: F-statistic with significance level of ANOVA and coefficient of multiple determination of multiple linear regression models to assess the association between the variation in the six CYP isoforms activity and in the three pro-inflammatory markers ($p < 0.05$ is significant)

	Model with Δ TNFα	**Model without Δ TNFα**
Δ CYP1A2	$F(3,24) = 3.299$, $p = 0.038$ $R^2 = 0.292$	$F(2,25) = 5.073$, $p = 0.014$ $R^2 = 0.289$
Δ CYP2B6	$F(3,24) = 0.500$, $p = 0.686$ $R^2 = 0.059$	$F(2,25) = 0.238$, $p = 0.790$ $R^2 = 0.019$
Δ CYP2C9	$F(3,24) = 2.447$, $p = 0.088$ $R^2 = 0.234$	$F(2,25) = 3.761$, $p = 0.037$ $R^2 = 0.231$
Δ CYP2C19	$F(3,24) = 1.329$, $p = 0.288$ $R^2 = 0.142$	$F(2,25) = 1.459$, $p = 0.252$ $R^2 = 0.105$
Δ CYP2D6	$F(3,24) = 3.774$, $p = 0.024$ $R^2 = 0.321$	$F(2,25) = 1.327$, $p = 0.283$ $R^2 = 0.096$
Δ CYP3A	$F(3,24) = 2.332$, $p = 0.100$ $R^2 = 0.226$	$F(2,25) = 3.642$, $p = 0.041$ $R^2 = 0.226$

Chapter 6: Influence of Inflammation on Cytochromes P450 Activity in Adults: A Systematic Review of the Literature.

Summary

As shown in **chapters 4** and **5**, acute inflammation has an impact on CYPs activities and inflammation is a potentially relevant criterion for CYPs expression and activity variabilities. However, inflammation is a condition encountered in many diseases, because it is a response to endogenous or exogenous aggression that can be either acute or chronic. In other words, the entire population will face it at least once in their lifetime because it is a universal protective response involving innate and adaptive immunity. *In vitro* and animal studies indicated that inflammation influences CYPs activity via several complex mechanisms at the transcriptional and post-transcriptional levels and through epigenetic modifications. These complex mechanisms could be different according to the sources of inflammation. Therefore, different impacts on CYPs activity than those observed in **chapter 4** (surgery) and **chapter 5** (SARS-CoV-2 infection) are expected, depending on the sources of inflammation.

Chapter 6 aims to review the current published data on the dynamic impact of inflammation on CYPs activity and expression in human adults. This systematic review (**review article 3**), published in *Frontiers of Pharmacology* included 218 studies and case reports, divided into 14 sources of inflammation. This drug-disease interaction had a significant impact on some CYPs substrates, but the effect appeared to be isoform-specific and related to the nature and the severity of the disease. Therefore, people with inflammation should be recognized as a special population and inflammatory state should be considered, in addition to the genotype and comedications of patients, to individualize treatments.

However, data are still scarce regarding resolution of inflammation (natural progression or secondary to treatment of initial disease or subsequent inflammation) and return to baseline CYPs activities. Moreover, **chapter 6** highlights that the use of a cocktail approach to assess the activity of the main CYPs simultaneously during inflammation is limited. Further development of the cocktail approach would provide data on all relevant CYPs found in humans. Indeed, **chapter 6** shows that studies have largely focused on CYP3A.

My contributions to this **review article 3** were the participation in the manuscript conceptualization, experimental design, systematic research, data analysis and writing the article.

Review article 3: Influence of Inflammation on Cytochromes P450 Activity in Adults: A Systematic Review of the Literature.

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Influence of Inflammation on Cytochromes P450 Activity in Adults: A Systematic Review of the Literature

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Background: Available in-vitro and animal studies indicate that inflammation impacts cytochromes P450 (CYP) activity *via* multiple and complex transcriptional and post-transcriptional mechanisms, depending on the specific CYP isoforms and the nature of inflammation mediators. It is essential to review the current published data on the impact of inflammation on CYP activities in adults to support drug individualization based on comorbidities and diseases in clinical practice.

Methods: This systematic review was conducted in PubMed through 7th January 2021 looking for articles that investigated the consequences of inflammation on CYP activities in adults. Information on the source of inflammation, victim drugs (and CYPs involved), effect of disease-drug interaction, number of subjects, and study design were extracted.

Results: The search strategy identified 218 studies and case reports that met our inclusion criteria. These articles were divided into fourteen different sources of inflammation (such as infection, autoimmune diseases, cancer, therapies with immunomodulator. . .). The impact of inflammation on CYP activities appeared to be isoform-specific and dependent on the nature and severity of the underlying disease causing the inflammation. Some of these drug-disease interactions had a significant influence on drug pharmacokinetic parameters and on clinical management. For example, clozapine levels doubled with signs of toxicity during infections and the concentration ratio between clopidogrel's active metabolite and clopidogrel is 48-fold lower in critically ill patients. Infection and CYP3A were the most cited perpetrator of inflammation and the most studied CYP, respectively. Moreover, some data suggest that resolution of inflammation results in a return to baseline CYP activities.

Conclusion: Convincing evidence shows that inflammation is a major factor to be taken into account in drug development and in clinical practice to avoid any efficacy or safety issues because inflammation modulates CYP activities and thus drug pharmacokinetics. The impact is different depending on the CYP isoform and the inflammatory disease considered. Moreover, resolution of inflammation appears to result in a normalization of CYP activity. However, some results are still equivocal and further investigations are thus needed.

Keywords: inflammation, cytochrome P450, pharmacokinetic, disease-drug interaction, cytokines

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INTRODUCTION

Cytochromes P450 (CYP) are the major drug-metabolizing enzymes (DME) responsible for 75% of drug metabolism, making them decisive in the efficacy and safety of drugs (Wienkers and Heath, 2005). The interindividual variability in CYP activity is influenced by genetic factors, environmental factors and comorbidities (Lynch and Price, 2007). CYP genetic polymorphisms are well described, resulting in major functional differences (Zhou et al., 2017). CYP are also impacted by drug-drug interactions (DDIs) and several widely used drugs were removed from the market because of serious adverse drug reactions (ADRs) due to DDIs via the CYPs (Wilkinson, 2005). Therefore, the Food and Drug Administration (FDA) requires *in-vitro* evaluation of potential DDIs during the course of drug development (Kato, 2020; Food and Drug Administration).

A less well described but increasingly studied source of modulation of CYP activity and recently reviewed is that of endogenous inflammatory markers (de Jong et al., 2020; Stanke-Labesque et al., 2020). Inflammation is a response to endogenous or exogenous aggression that can be acute or chronic. It is prominent in many diseases, such as infection, trauma, surgery, arthritis, asthma, atherosclerosis, autoimmune disease, various immunologically mediated and crystal-induced inflammatory conditions, diabetes and cancer, to name a few (Gabay and Kushner, 1999; Germolec et al., 2018; Stavropoulou et al., 2018). This universal protective response involves innate and adaptative immunity and is present in virtually all tissues. Acute changes can be associated with variation in the concentrations of several plasma proteins, the acute-phase proteins (APP), and numerous behavioral, physiological, biochemical and nutritional changes (Gabay and Kushner, 1999). Cytokines are the main stimulators of APP production, and interleukin-6 (IL-6) is the key stimulator of APP while other cytokines (IL-1β, Tumor Necrosis Factor α, interferon-γ, transforming growth factor β and possible IL-8) influence APP subgroups (Gabay and Kushner, 1999). Thus, inflammation is a complex and well-orchestrated process involving many cell types and molecules that function as a cascade network, some of which initiate, amplify or sustain the process and others attenuate or resolve it (Gabay and Kushner, 1999; Stanke-Labesque et al., 2020).

Inflammation can impact drug PK through multiple mechanisms which typically occur in the liver, kidney, or intestinal epithelial cells (Stavropoulou et al., 2018; de Jong et al., 2020; Stanke-Labesque et al., 2020). The metabolic activities of CYPs are suppressed by inflammation in most cases, but some CYPs may be induced or remain unaffected (Morgan, 2001; de Jong et al., 2020; Stanke-Labesque et al., 2020). The positive and negative control of gene transcription is generally achieved by the interaction of regulatory proteins with specific DNA sequences on the regulated genes (Morgan, 1997). The impact of inflammation on the metabolic activity of CYPs has been studied in various *in-vitro* and animal models of inflammation, including trauma, infection and administration of endotoxin or cytokines (de Jong et al., 2020; Stanke-Labesque et al., 2020). Information available in the literature suggests that this impact on PK is triggered by cytokines and their intracellular signaling, directly or *via* interaction with the nuclear receptor pathway, on drug transporters and metabolizing enzymes (Liptrott and Owen, 2011; de Jong et al., 2020; Stanke-Labesque et al., 2020). Importantly, no single common pathway has been identified to explain the changes in the entire CYP family and involves different mediators but also different transcription factors (Renton, 2005; de Jong et al., 2020; Stanke-Labesque et al., 2020). Different effects of cytokines are observed in different cell types, which could be explained by a difference in the way intracellular signals from cytokine receptors are generated (Liptrott and Owen, 2011). Different cytokines exhibit a widely different spectrum of activity trough individual CYP isoforms and many different transcription factors (Morgan, 1997; Ruminy et al., 2001; Renton, 2005; Liptrott and Owen, 2011). Their activation by cytokines have been implicated in the downregulation and transcriptional regulation of different CYP isoforms (Morgan, 1997; Ruminy et al., 2001; Renton, 2005; Liptrott and Owen, 2011). Regulation of CYP during inflammation can occur trough pre- and post-transcriptional mechanisms that are cytokine and CYP specific (de Jong et al., 2020; Stanke-Labesque et al., 2020). Pre-transcriptional mechanisms currently described in the literature include transcriptional downregulation of transcription factors, interference with dimerization/translocation of (nuclear) transcription factors, altered liver-enriched C/EBP signaling, and direct regulation by NF-κB (de Jong et al., 2020). Overall, three main mechanisms have been described to explain the downregulation of inflammation in drug metabolizing enzyme and transporters expression and activity, namely inhibition of drug metabolizing enzyme transcription, epigenetic modifications in genes as a result of DNA methylation, modification of histone patterns, release of microRNA and NO-dependent proteasome degradation, which is a post-transcriptional mechanism (Stanke-Labesque et al., 2020).

Therefore, the aim of this systemic review is to evaluate the impact of inflammation on CYP activity in the adult population.

METHODS

The method used to manage the literature search was based on the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement (Moher et al., 2009). The detailed PICOS framework (i.e., participants, interventions, comparisons, outcomes, study design) was used as follows: Participants: adults with source of inflammation, -Intervention: victim drugs and CYPs concerned, -Comparison: healthy adults or before the onset of inflammation or receiving treatment for inflammation Outcomes: potential effect of interaction between inflammation and CYP activity, -Study design: clinical trials and case reports/series.

Database and Search Strategy

The literature search was performed in PubMed via MEDLINE, the database of biomedical publications, for studies and case reports/series until January 7, 2021. To expand it, we also

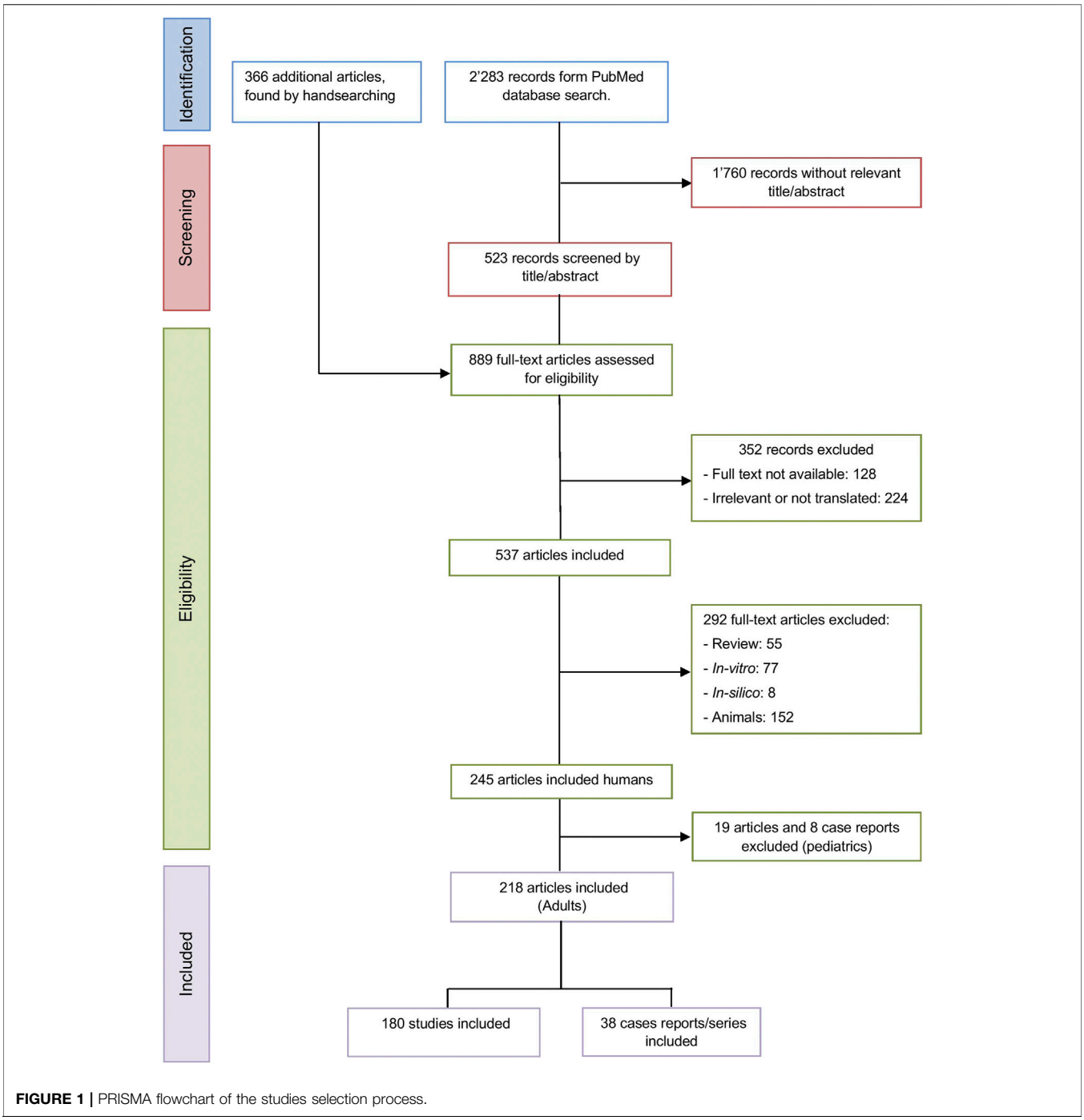

FIGURE 1 | PRISMA flowchart of the studies selection process.

performed a manual search of references for potentially relevant articles. The keywords used were "inflammation", "cytochrome P450", "cytochromes P450" and "CYP450."

Study Selection

We applied the eligibility criteria described below in order to filter relevant publications from the total of results provided by the literature search.

The types of studies included in our literature search were randomized controlled trials, non-randomized studies, and observational studies, including case reports and series, published as full-text articles and congress abstracts in English. The year of publication selected was from database inception until January 7, 2021. Study participants had to be older than 18 years old, including healthy subjects and patients with an inflammatory

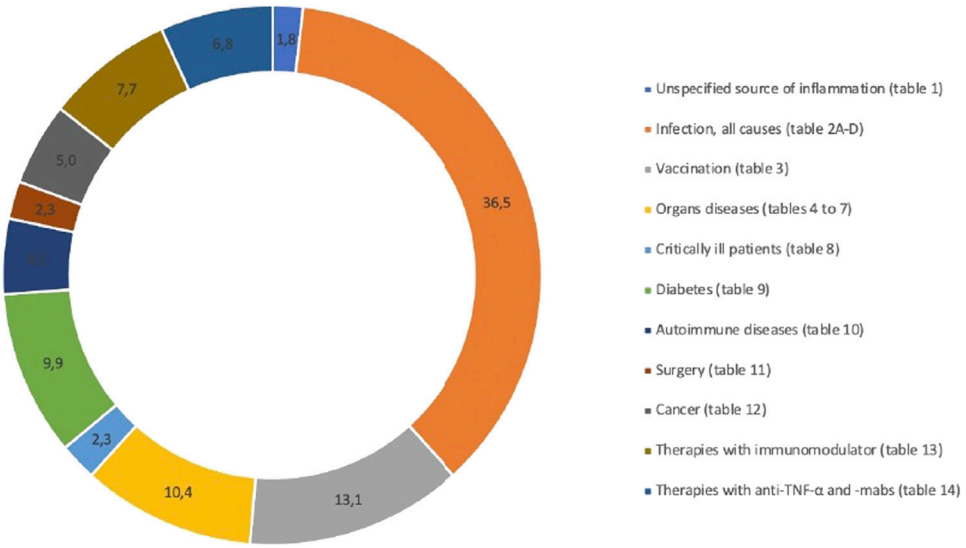

FIGURE 2 | Distribution (%) of included references according to the different sources of inflammation.

condition, caused by disease, treatment or a medical or surgical procedure. The outcomes of interest were the effect of potential inflammation (suggested or provided) on metabolic ratios (MR) of CYP isoforms, the PK/PD and the safety profile of CYP substrates.

Successive steps in article selection included reading the title, abstract and full text according to the predefined eligibility criteria to screen for potentially relevant records. The selected articles were classified into literature reviews and *in-vitro*, animal, *in-silico* and human studies. Then, only studies involving adults (defined as over 18 years old) were kept, classified into studies or case reports/series. The same procedure was applied to assess the inclusion of additional articles identified by the manual search. The study selection process was summarized in a flowchart created according to the PRISMA statement requirements (**Figure 1**) (Moher et al., 2009).

Data Extraction and Management

Articles selected from the search results were collected and exported to the reference management software Zotero (version 5.0.85, © 2006–2018 Contributors) and merged to remove duplicates. Data from the included articles were extracted and synthetized. The authors extracted the following data according to the PICOS framework discussed above. These included study design, sample size, source of inflammation and comparators, victim drugs and CYP involved, and outcomes of interests (potential effect of interaction). When a CYP substrate was used in the article to determine whether or not inflammation or concomitant drugs altered its PK/PD profile, a verification of its metabolic pathway was performed. The verification process was performed using the Summary of Product Characteristics (SmPCs), the Lexi-Interact drug interaction checker and the Geneva table of CYP substrates, inhibitors, and inducers (Uptodate,; Samer et al., 2013).

RESULTS

Identification and Selection of the Studies

The primary search, performed in PubMed, yielded a total of 2′283 articles that were screened according to their title and abstract. Of the remaining 523 articles, an additional 366 articles were identified by cross-referencing and handsearching of the reference list of the relevant articles (n = 889). Of these, 352 records were removed because the full text was not available (n = 128) or because they were considered irrelevant or not translated into English (n = 224). The remaining 537 articles were classified into review articles (n = 55), *in-vitro* (n = 77) or *in-silico* (n = 8) studies, and animal (n = 152) or human (n = 245) studies. The articles and case reports concerning the pediatric population (n = 27) are the subject of another systematic review and were excluded from this work (Lenoir et al., 2021). Finally, 218 articles conducted in adults were included and classified into studies (n = 180) and case reports/series (n = 38) for analysis (**Figure 1**).

Results of the Studies

The 218 eligible publications are summarized in **Table 1** through 14. The drug-disease interactions found in the selected articles were divided into fourteen different sources of inflammation: unspecified source of inflammation (**Table 1**), infection (**Table 2A**), infection-example hepatitis (**Table 2B**), infection-example HIV (**Table 3C**), infection-example SARS-CoV-2 (**Table 2D**), vaccination (**Table 3**), kidney disease (**Table 4**), liver disease (**Table 5**), lung disease (**Table 6**), heart disease (**Table 7**), critically ill patients (**Table 8**), diabetes (**Table 9**), autoimmune diseases (**Table 10**), surgery (**Table 11**), cancer (**Table 12**), therapies with immunomodulator (**Table 13**) and therapies with anti-TNF-α and -mabs (**Table 14**). The most cited inflammation perpetrator was infection and the most studied CYP was CYP3A. CYP3A subfamilies refers to CYP3A4 and

TABLE 1 | Impact of unspecified source inflammation on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
IL-10 injection	tolbutamide (CYP2C9), caffeine (CYP1A2), dextromethorphan (CYP2D6) and midazolam (CYP3A)	12	- significantly but moderately decreased CYP3A4 activity (12 ± 17%, $p < 0.02$) - significantly increased CYP2C9 activity (38 ± 25%, $p < 0.005$), - no significant changes in either CYP1A2 or 2D6 activity	Wienkers and Heath (2005) Double-blind crossover study
Elevated CRP levels (>1.5 mg/dl)	perampanel (CYP3A4)	111 = Total 23 = CRP>1.5 mg/dl 13 = enzyme-inducing AEDs 10 = no enzyme-inducing AEDs	- perampanel C/D increased by 53.5 and 100.8% respectively when CRP >1.5 mg/dl - correlation between serum CRP level and C/D of perampanel ($r = 0.44$, $p < 0.001$)	Lynch and Price (2007) Cohort study
Erythrocyte sedimentation rate (ESR) > 20 mm vs. control	Oxprenolol (CYP2C9, 2D6, 3A4 and 1A2 substrate)	18	- mean oxprenolol AUC 2-fold greater in inflammation group	Zhou et al. (2017) Cohort study
CRP serum levels	tacrolimus (CYP3A4)	31-year-old man	-tacrolimus C/D increased during two inflammation episodes by 54% (cholestasis) and 141% (infection following surgery), and strongly correlated with CRP (r2 = 0.78, $p = 0.079$)	Wilkinson (2005) case report

CYP3A5, because the probe drugs used to assess the activity of CYP3A4 are metabolized by these two isoenzymes and no distinction can be made between them. Distribution in percent of all the references in the different categories are illustrated in **Figure 2**.

Infection

Several studies have assessed the association between infection, represented by elevated levels of CRP, and PK variations of voriconazole. This is of particular interest and voriconazole therapeutic drug monitoring should thus be used to optimize clinical success and safety in these settings (Luong et al., 2016). Increased levels of CRP were correlated with increased voriconazole concentrations or decreased metabolic ratio of voriconazole/N-oxide and this could be explained by CYP2C19 and/or CYP3A downregulation, as voriconazole is mainly metabolized by these two CYPs (van Wanrooy et al., 2014; Encalada Ventura et al., 2015; Dote et al., 2016; Niioka et al., 2017; Vreugdenhil et al., 2018; Schulz et al., 2019). A positive correlation between inflammatory markers and voriconazole concentration was seen in adults, as well as with the severity of infection (van Wanrooy et al., 2014; Dote et al., 2016; Veringa et al., 2017; Gautier-Veyret et al., 2019). Drug metabolism appears to be influenced by the degree of inflammation and standardization of the classification of inflammatory markers elevation seems necessary (van Wanrooy et al., 2014; Niioka et al., 2017; Veringa et al., 2017; Gautier-Veyret et al., 2019). Indeed, voriconazole through concentration increased by 0.015 mg/L every 1 mg/L increase in CRP, and a recent meta-analysis showed that an increase in voriconazole through concentration of 6, 35 and 82% was associated with an increase in the CRP level of 10, 50 and 100 mg/L, respectively (van Wanrooy et al., 2014; Bolcato et al., 2021). As a final evidence to support of a correlation between inflammation and CYP downregulation, inflammation, and its resolution, decreased, and increased voriconazole clearance respectively, suggesting that the improvement of the inflammation allows a return to the baseline (Dote et al., 2016). However, no studies have investigated the duration of the resolution of inflammation-induced metabolic phenoconversion (Stanke-Labesque et al., 2020). This is an important limitation to allow individualization of treatment without therapeutic drug monitoring (TDM), as under-exposure to drug remains a risk (Stanke-Labesque et al., 2020).

CYP downregulation was also demonstrated as a consequence of sufficient inflammation and significant temperature elevation (Elin et al., 1975). Therefore, caution should be exercised in case of infection when administering CYP substrates, as this may result in toxicity and ADRs (Vozeh et al., 1978; Blumenkopf and Lockhart, 1983; Levine and Jones, 1983 1; Raaska et al., 2002; Haack et al., 2003; de Leon and Diaz, 2003; Jecel et al., 2005; Darling and Huthwaite, 2011; Espnes et al., 2012; Kwak et al., 2014; Leung et al., 2014; Takahashi et al., 2015; Clark et al., 2018; Khan and Khan, 2019).

Early works assessed the effect of an infection induced intentionally by lipopolysaccharides (LPS) injection on antipyrine pharmacokinetics, and several studies have assessed the impact of infection on psychotropic agents (clozapine, risperidone). The increase of clozapine levels, a CYP1A2 substrate, due to inflammation has been well studied and demonstrated (Raaska et al., 2002; Haack et al., 2003; de Leon and Diaz, 2003; Jecel et al., 2005; Pfuhlmann et al., 2009; Darling and Huthwaite, 2011; Espnes et al., 2012; Abou Farha et al., 2012; Leung et al., 2014; Kwak et al., 2014; Takahashi et al., 2015; ten

TABLE 2A | Impact of infection on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Lipopolysaccharides (LPS)-induced inflammation	theophylline (CYP1A2), hexobarbital (CYP2C19) and antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	12	- significant repression of CYPs activity (takes several hours to develop)	Kato (2020), Crossover study
Two injections of Gram-negative bacterial endotoxin	theophylline (CYP1A2), hexobarbital (CYP2C19) and antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	9	- significant decrease of clearances of all probes compared with the saline control studies, - endotoxins injections associated with decreased hepatic drug metabolism, mainly CYP1A2 and 2C19	Food and Drug Administration, Cross-over clinical trial
Administration of a single oral dose of 10 mg/kg of etiocholanolone	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	14 = significant fever (fever index >50)	- half-life was significantly prolonged (29.3%, $p < 0.005$) in patients with significant fever	de Jong et al. (2020)
		19 = failed to develop significant fever (fever index <50)	- no significant change of half-life ($p > 0.8$) in patients without significant fever	Cross-over clinical trial
			- no correlation between the magnitude of fever and the extent to which half-life was prolonged	
Acute pneumonia	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	14	- 1.5 fold increased clearance 14 and 28 days after the acute illness - enhancement of clearance in 28 days represented a 36% improvement	Stanke-Labesque et al. (2020) Cohort study
Liver fluke infection (uninfected, infected only and infected with fibrosis)	coumarine (CYP2A6)	- Total = 91	- 26% lower urine levels of 7-hydroxycoumarine (7-HC) after praziquantel ($p < 0.001$) compared to initial assessment	Stavropoulou et al. (2018)
		- 73 completed the two assessments	- infected individuals excreted slightly higher levels of 7-HC in the 0–2 h period	Cohort study
Herpes zoster	warfarin (CYP2C9)	66-year-old woman	- acute spinal subdural hematoma and subarachnoid haemorrhage during the course of a thoracic level infection - 3-fold increased PT times requiring vitamin K administration	Germolec et al. (2018) Case report
Visceral leishmaniasis	midazolam (CYP3A), omeprazole (CYP2C19), losartan (CYP2C9)	24	- significantly increased midazolam CL/F ($p = 0.018$) 2–3 days and 3–6 months after curative chemotherapy - significantly increased omeprazole CL/F ($p = 0.008$) 2–3 days and 3–6 months after curative chemotherapy - CYP2C9 activity not significantly different between	Gabay and Kushner (1999) Cohort study
Influenza A	theophylline (CYP1A2)	50-year-old woman	- toxicity symptoms after infection - increased theophylline levels (1.5x above normal values)	Morgan (2001) Case report
Acute illness	theophylline (CYP1A2)	3	- 2-fold or 3-fold variation in clearance during acute illness - clearance decreased during worsening of airway obstruction in one patient - 2 patients had increased clearance during the improvement of their condition (pneumonia and congestive heart failure)	Morgan (1997) Case series
Elevated CRP levels (>5 mg/L) vs control	citalopram (major CYP2C19, minor CYP3A4) and venlafaxine (major CYP2D6, minor CYP3A4 and 2C19)	15 citalopram	- no statistical differences in citalopram and venlafaxine concentrations or in MR of both drugs in samples with elevated CRP levels	Liptrott and Owen (2011)
		39 venlafaxine		Cohort study
Elevated serum levels of CRP	risperidone (bioactivated by CYP3A4 and CYP2D6)	2 females (56 and 38 years old)	- close temporal association between serum levels of risperidone active moiety (risperidone + 9-hydroxyrisperidone) and CRP - > 3x increase of C/D during elevated CRP serum concentration	Renton (2005) Case report

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TABLE 2A | (*Continued*) Impact of infection on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
			- parallel fluctuation of drug levels and CRP which necessitated dose adjustments, but the MR was unchanged, suggesting that the CYP2D6-catalyzed formation of 9-hydroxyrisperidone was not affected	
Pneumonia	risperidone (bioactivated by CYP3A4 and CYP2D6)	56-year-old man	5-fold higher risperidone dose requirement during pneumonia	Ruminy et al. (2001)
				Case report
Elevated serum levels of CRP (>5 mg/L)	clozapine (CYP1A2), quetiapine (CYP3A4 and CYP2D6) and risperidone (CYP3A4 and CYP2D6)	33 clozapine, 32 quetiapine 40 risperidone	- C/D of clozapine was significantly higher ($p < 0.01$) and CYP1A2 MR (NCLZ/CLZ) significantly lower ($p < 0.05$)	Moher et al. (2009)
			- positive and significant correlation between clozapine and CRP levels ($r = 0.313$, $p < 0.01$) - no difference in C/D or in MR of quetiapine - C/D of risperidone was significantly higher ($p < 0.01$) and MR decreased (NS)	Cohort study
Elevated serum levels of CRP	clozapine (CYP1A2)	27 high drug level	mean CRP value significantly higher ($p = 0.005$) in patients with elevated clozapine level	Uptodate
		36 normal drug level		Case-control study
Elevated serum level of CRP of 130 mg/L	clozapine (CYP1A2)	44-year-old man	- admission to hospital because of symptoms of clozapine toxicity - elevated clozapine levels - condition improved when treatment was discharged	Samer et al. (2013) Case report
Elevated serum level of CRP of 256 mg/L	clozapine (CYP1A2)	50-year-old man	- 5-fold increased plasma levels 4 days after admission	Lenoir et al. (2021)
				Case report
Sepsis	clozapine (CYP1A2)	61-year-old woman	- clozapine toxicity symptoms - increased clozapine serum levels = 4318 ng/ml (References = 350–700 ng/ml)–All patients improved after dose reductions	Luong et al. (2016) Case reports
Suspected infections	clozapine (CYP1A2)	4	- clozapine toxicity symptoms in usually stable patients - patients improved after dose reduction or therapy discontinuation	Dote et al. (2016) Case series
Suspected infections	clozapine (CYP1A2)	62-year-old man	- clozapine levels increased during infection (from 377 ng/ml to 1′628 ng/ml)	Encalada Ventura et al. (2015)
				Case report
Respiratory infection	clozapine (CYP1A2)	34-year-old man	- increased clozapine levels to 1245 ng/ml during infection	Niioka et al. (2017)
				Case report
Lung abscess	clozapine (CYP1A2)	29-year-old man	- increased clozapine levels during infection (from 681 ng/ml to 1′467 ng/ml) - No signs of clozapine toxicity	Encalada Ventura et al. (2015) Case report
Influenza A	clozapine (CYP1A2)	33-year-old woman	- increased clozapine levels during infection (from 661 ng/ml to 1′300 ng/ml) - symptoms of clozapine toxicity	Encalada Ventura et al. (2015) Case report
Pneumonia	clozapine (CYP1A2)	42-year-old man	- increased clozapine levels during infection (from 1′024 ng/ml to 2′494 ng/ml) - symptoms of clozapine toxicity	Encalada Ventura et al. (2015) Case report
Pneumonia	clozapine (CYP1A2)	35-year-old man	- increased median clozapine C/D ratios at the peak of infection	Vreugdenhil et al. (2018)
				Case report
Upper respiratory tract infection	clozapine (CYP1A2)	68-year-old woman	- increased clozapine levels during infection (peaked at 1′096 ng/ml) - toxicity symptoms	van Wanrooy et al. (2014) Case report

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TABLE 2A | (*Continued*) Impact of infection on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Upper respiratory tract infection	clozapine (CYP1A2)	47-year-old man	- On day 24 and 25 (highest level of infection severity), serum concentration levels increased to 881.2 and 663.5 ng/ml, respectively	Schulz et al. (2019) Case report
Urinary tract infection	clozapine (CYP1A2)	51-year-old woman	- increased clozapine levels during infection (peak at 1′066 ng/ml) - patients improved after dose reduction and recovery	Veringa et al. (2017) Case report
Urinary tract infection	clozapine (CYP1A2)	45-year-old woman	- increased clozapine levels during infection (from 705 ng/ml to 2′410 ng/ml) - toxicity symptoms	Encalada Ventura et al. (2015) Case report
Urinary tract infection	clozapine (CYP1A2)	62-year-old man	- increased clozapine levels during infection (from 432 ng/ml to 1′192 ng/ml) - no toxicity symptoms	Encalada Ventura et al. (2015) Case report
Urinary tract infection	clozapine (CYP1A2)	64-year-old woman	- decreased clozapine levels after infection recovery (from 749.4 to 260.0 ng/ml) - toxicity symptoms	Gautier-Veyret et al. (2019) Case report
Infections	clozapine (CYP1A2)	16 patients with 18 episodes	- only 2 episodes did not require any relevant changes of dosage	Bolcato et al. (2021) Case series
Infections	clozapine (CYP1A2)	3	- clozapine toxicity symptoms - 2.5-7-fold increased clozapine serum concentration during infections	Elin et al. (1975) Case series
Diarrheic stools and gastrointestinal bacterial infection	clozapine (CYP1A2)	23 years old man	- at admission, CRP serum concentration = 130 mg/ml and clozapine serum concentration = 9074 nmol/L (References interval 200–2500 nmol/L) - 1 month before, serum concentration = 1919 nmol/L 1 month before admission and fairly constant during the last years	Blumenkopf and Lockhart (1983) Case report
Bacterial pneumonia	clozapine (CYP1A2)	53-year-old woman	- trough concentration = 2074 µg/L at day 0 (before any antibiotics treatments) - previous trough concentrations were three times lower - during the infection, CRP = 152 mg/L and α1-glycoprotein = 2398 mg/L - concentration decreased nearly to the previous levels after 2 weeks (624 ± 214 mg/L)	Khan and Khan (2019) Case report
Increased CRP level	voriconazole (CYP3A4 and CYP2C19)	63	- increased CRP levels associated with significantly increased voriconazole C/D ($p < 0.05$) - CYP3A4 and CYP2C19 downregulated by inflammation	Vozeh et al. (1978) Retrospective study
Increased CRP level	voriconazole (CYP3A4 and CYP2C19)	19	- inflammatory response positively associated with voriconazole concentration ($r = 0.62$, $p < 0.001$) - inflammatory response negatively associated with voriconazole MR (rho = -0.64, $p < 0.001$)	Cohort study Leung et al. (2014) Cohort study
Elevated CRP level	voriconazole (CYP3A4 and CYP2C19)	54	- voriconazole/N-oxide ratio could be predicted by the CRP concentration with a standardized regression coefficient of 0.380 ($p = 0.001$)	Haack et al. (2003) Cohort study
Elevated IL-6, IL-8 and CRP levels	voriconazole (CYP3A4 and CYP2C19)	22	- correlation between IL-6 ($r = 0.46$, $p < 0.0001$), IL-8 ($r = 0.42$, $p < 0.0001$) and CRP ($r = 0.53$, $p < 0.0001$) and trough concentration	de Leon and Diaz (2003) Cohort study

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TABLE 2A | (*Continued*) Impact of infection on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
CRP serum level	voriconazole (CYP3A4 and CYP2C19)	Total = 128	- trough concentration increased by 0.015 mg/L every 1 mg/L increase in CRP	Jecel et al. (2005)
- Elevated (>200 mg/L)			- correlation between trough concentration and CRP levels ($p < 0.001$), and with severity of inflammation	Retrospective study
- Moderate (>41 mg/L, <200 mg/L)				Cohort study
- Control (<40 mg/L)				
Multiple infections along his 5 months hospital stay	voriconazole (CYP2C19 and 3A4), meropenem and their combinations	78-year-old man	- decreased voriconazole dose requirements	Darling and Huthwaite (2011)
				Case report
CRP serum level	voriconazole (CYP3A4 and CYP2C19)	34	- MR significantly decreased with higher CRP concentration after adjustment ($p < 0.001$)	Espnes et al. (2012)
		20 = patients with CYP2C19 genotype performed	- extent of decrease of MR and increase of trough concentration varied between the different genotypes ($p < 0.001$ and $p = 0.04$, respectively)	Prospective study
CYP2C19 genotype				Cohort study
CRP serum levels	voriconazole (CYP3A4 and CYP2C19) and itraconazole (CYP3A4)	41 voriconazole	- C/D of voriconazole and of voriconazole N-oxide positively (r = 0.61, $p < 0.01$) and negatively (r = -0.52, $p < 0.01$) correlated with CRP levels, respectively	Raaska et al. (2002)
		42 itraconazole	- C/D of itraconazole ($p = 0.33$) and its hydroxide ($p = 0.52$) were not correlated with CRP	Cohort study
CRP serum levels	voriconazole (CYP3A4 and CYP2C19)	31 = with overdose	- mean CRP level significantly higher ($p < 0.0001$) in patients who experienced an overdose (188 mg/L) compared to those who did not (37 mg/L)	Levine and Jones (1983 1)
		31 = without overdose	- patients with CRP levels >96 mg/L (median level) had a 27-fold higher risk of overdose than patients with CRP levels <96 mg/L	Case-control study
Inflammation level	voriconazole CYP2C19 and 3A4)	64-year-old man	- voriconazole C/D associated with inflammation level	Clark et al. (2018)
				Case report
Influenza-like illness	phenytoin (CYP2C9 and CYP2C19 substrates and induces CYP2C9, 2C19 and 3 A)	52-years-old woman	- became increasingly drowsy, moody, complaining of staggering, difficulty to talking and visual disturbance with toxic phenytoin levels (51 μg/ml)	Kwak et al. (2014)
				Case report
Pneumonia	perampanel (CYP3A4)		- 3.5-fold increase perampanel concentrations, - reversible within 7 days after CRP normalization	Lynch and Price (2007)) Case report
Inoculation of Malaria	quinine (CYP3A4)	5	- increase quinine MR during infection ($p < 0.01$)	Takahashi et al. (2015)
				Cross-over study
Infection disease state (pneumonia, endocarditis, wound infection or gastroenteritis) vs healthy state	bisoprolol (CYP2D6 and 3A4) and nitrendipine (CYP3A4)	20	- PK parameters of bisoprolol unchanged ($p > 0.05$)	Hefner et al. (2016)
			- bioavailability of S-enantiomer twice that of R-nitrendipine in infection ($p < 0.01$) - 2-fold increased AUC and Cmax of S-nitrendipine ($p = 0.010$ and $p = 0.012$ respectively) and R-nitrendipine ($p = 0.005$ and $p = 0.029$)	Cohort study
Enteritis with diarrhoea	tacrolimus (CYP3A)	52	- mean tacrolimus trough level 2.3 times higher during enteritis ($p = 0.0175$)	Pfuhlmann et al. (2009)
			- mean trough level returned to their baseline levels 2 weeks after onset	Cohort study

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TABLE 2A | (*Continued*) Impact of infection on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Helicobacter pylori infection in cirrhotic patients	/	21 tested positive and 11 not	Hp-infected cirrhotic patients had a significant lower mean of the monoethylglycinexylide (MEGX) test compared to non-infected patients (p = 0.006), while 13C-galactose breath test (GBT) was not	Abou Farha et al. (2012)
Sepsis	tacrolimus (CYP3)	41-year-old man	151% increased tacrolimus C/D during sepsis	Case-control study Wilkinson (2005)
Dermatitis	clozapine (CYP1A2)	57-year-old woman	- On days 36 and 43 (highest level of dermatitis severity), clozapine serum concentration increased to 889.2 and 1′012 ng/ml, respectively	Case report Schulz et al. (2019) Case report

Bokum et al., 2015; Hefner et al., 2016; Ruan et al., 2017; Clark et al., 2018; Ruan et al., 2018; Ruan et al., 2020). A positive and significant correlation between clozapine and CRP levels (r = 0.313, $p < 0.01$) was found, with a 2- to 6-fold increase in serum levels and the development of toxic symptoms, as well as improvement after dose reduction or infection recovery (Raaska et al., 2002; Haack et al., 2003; de Leon and Diaz, 2003; Jecel et al., 2005; Pfuhlmann et al., 2009; Darling and Huthwaite, 2011; Espnes et al., 2012; Kwak et al., 2014; Leung et al., 2014; Takahashi et al., 2015; ten Bokum et al., 2015; Hefner et al., 2016; Abou Farha et al., 2012; Ruan et al., 2017; Clark et al., 2018; Ruan et al., 2018; Ruan et al., 2020). Further investigations are needed concerning anticoagulant therapy, as only one case of severe bleeding in the context of infection was reported in the literature (Blumenkopf and Lockhart, 1983). First observation of a return to baseline metabolic activity after the end of the disruption that caused inflammation dates from 1985, with the gradual improvement of antipyrine clearance in days after the resolution of pneumonia (Sonne et al., 1985). Later, other authors demonstrated metabolic recovery after improvement of a liver fluke infection following praziquantel treatment (Satarug et al., 1996).

In hepatitis (**Table 2B**), a study suggested an overall downregulation of several hepatic CYPs and transporters with liver fibrosis progression, although the mechanisms of regulation differed and large inter-individual variation existed (Hanada et al., 2012). Indeed, this study assessed that the mRNA level was largely dependent on fibrosis stage and that the role of the different nuclear receptors tested is not the same in the hepatic expression of each CYP isoenzyme (Hanada et al., 2012). CYP3A4 downregulation during HCV infection has been well-described (McHorse et al., 1975; Tuncer et al., 2000; Latorre et al., 2002; Wolffenbüttel et al., 2004). Indeed, numerous studies have described a higher drug exposure of the two most commonly used immunosuppressants, tacrolimus and cyclosporine A, in patients with hepatitis and especially in those with viremia (Tuncer et al., 2000; Latorre et al., 2002; Wolffenbüttel et al., 2004). Moreover, when HCV is treated, CYP activities appear to return to baseline levels in several studies (McHorse et al., 1975; van den Berg et al., 2001; Kugelmas et al., 2003; Ueda et al., 2015; Kawaoka et al., 2016; Saab et al., 2016; Raschzok et al., 2016; Ueda and Uemoto, 2016; Smolders et al., 2017). Indeed, through concentration of tacrolimus decreased after initiation of HCV treatment, such as sofosbuvir, daclatasvir, asunaprevir, simeprivir, ribavirin and interferon, administered alone or in combination, and it required a dosage increase (Kawaoka et al., 2016; Raschzok et al., 2016; Saab et al., 2016; Smolders et al., 2017). Subgroups were identified, such as patients not responding to interferon with higher CYP3A downregulation related to higher levels of circulating cytokines, confirming that CYP modulation is proportional to intensity of inflammation (Morcos et al., 2013). However, conflicting results exist, and clinical recovery from acute liver disease was not accompanied by a corresponding recovery of drug-metabolizing capacity in a study (Breimer et al., 1975). This could be due to a lag between the return to baseline CYP levels and recovery, as clinical recovery from liver disease is not accompanied by a corresponding recovery of drug metabolizing capability (Breimer et al., 1975). Indeed, it is generally recognized that recovery half-lives are approximatively 20–50 h after mechanism-based inhibition and 40–60 h after enzyme induction (Imai et al., 2011).

Several studies have examined the impact of HIV on CYP metabolism (**Table 2C**) and have shown that several concomitant treatments and antiretroviral drugs metabolized by CYP3A have reduced metabolism in HIV-infected individuals, with an increased risk of ADRs. For instance, clindamycin clearance decreased from 0.27 in healthy volunteers to 0.21 L/h/kg in AIDS patients (p = 0.014) and a negative correlation between TNF-α and midazolam clearance was found (Gatti et al., 1993; Jones et al., 2010). Moreover CYP3A inhibitor (ketoconazole or ritonavir) and inducer (rifampicin) effects were less pronounced on antiviral PK in HIV-patients (Gatti et al., 1993; Grub et al., 2001; Jetter et al., 2010; European medicines agency; Packageinserts). It is important to characterize CYP3A modulation in HIV, as many antiviral treatments are metabolized by this pathway, and this could lead to efficacy or safety concerns. However, the AUC of atazanavir was lower in HIV-infected patients than in healthy volunteers and this could

TABLE 2B | Impact of hepatitis on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Chronic hepatitis C	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	12 = chronic hepatitis C 18 = controls	- decreased clearance and greater excretion in urine (about 50%, $p < 0.01$) - no difference in hepatic enzymes levels but Child Pugh Score correlated with clearance ($r = -0.73$, $p = 0.007$)	ten Bokum et al. (2015) Case-control study
Chronic hepatitis C	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	85	- no difference in clearance before and after 6 weeks of interferon treatment - 14% clearance increased ($p < 0.05$) 6 months later among responders but not in those who had failed to respond to interferon	Ruan et al. (2017) Cohort study
Acute viral hepatitis	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	6	- decreased plasma half-life and plasma clearance during the acute phase of hepatitis compared to recovery period ($p < 0.02$)	Ruan et al. (2018) Cohort study
Acute hepatitis	hexobarbital (CYP2C19)	13 = hepatitis 14 = controls	- decreased elimination half-life in patients with hepatitis compared to controls (490 ± 186 min vs. 261 ± 69 min, $p < 0.001$)	Ruan et al. (2020) Case-control study
Hepatitis C infection (IFN)	Cyclosporin A (CyA) and tacrolimus (CYP3A4)	26 = hepatitis C infection 78 = controls	- Lower doses ($p < 0.05$) in hepatitis C as compared to controls, while levels were comparable	Sonne et al. (1985) Case-control study
Acute viral hepatitis C	CyA (CYP3A4)	18 = HCV Ab + 18 = HCV Ab -	- CyA levels significantly higher in HCV Ab + ($p = 0.0001$)	Satarug et al. (1996) Case-control study
Acute viral hepatitis C	CyA (CYP3A4)	11 = anti-HCV + 11 = controls	- altered CyA PK (higher peak levels and drug exposure) in HCV+, especially those with viremia	Hanada et al. (2012) Case-control study
Acute viral hepatitis C	CyA (CYP3A4)	10 = anti-HCV + 14 = controls	- CyA AUC 69% ($p < 0.01$) and 32% ($p < 0.01$) higher in pre- et post-transplant studies in HCV + patients	Hanada et al. (2012) Case-control study
Acute viral hepatitis	meperidine (CYP2B6, 2C19 and 3A4)	14 = acute viral hepatitis 15 = controls	- terminal plasma half-life significantly prolonged in acute viral hepatitis compared to controls ($p < 0.001$) and 2-fold change in total plasma clearance observed ($p < 0.002$)	Latorre et al. (2002) Case-control study
Acute viral hepatitis	meperidine (CYP2B6, 2C19 and 3A4)	5	- total plasma clearance increased from 488 ± 132 ml/min to 1200 ± 555 ml/min and the terminal half-life decreased from 8.24 ± 3.71 to 3.25 ± 0.80 h respectively ($p < 0.005$) - values after recovery were not significantly different from those of the control group	Latorre et al. (2002) RCT
Chronic hepatitis C (CHC)	midazolam (CYP3A4)	107 = controls 35 = CHC naïve to treatment 24 = CHC null responders to IFN	- MR decreased by 37 and 54% ($p < 0.05$) in patients with hepatitis C treatment-naive and interferon null-responders respectively, compared to controls - consistent reductions in CYP3A4 activity between healthy volunteers and patients infected, most substantial difference with interferon null-responders	Tuncer et al. (2000) Case-control study
liver kidney microsome type 1 (LKM-1) antibodies	dextromethrophan (CYP2D6)	10 negative and 10 positive patients for LKM-1	- dextromethorphan-to-dextrorphan (DEM/DOR) ratio was significantly higher in liver kidney microsome type (LKM-1) positive patients ($p = 0.004$), showing that CYP2D6 activity had decrease (antibodies are targeted against CYP2D6)	Wolffenbüttel et al. (2004) Case-control study

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TABLE 2B | (*Continued*) Impact of hepatitis on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Hepatitis A	coumarine (CYP2A6)	9 = hepatitis A	- mean reduction of 37% ($p < 0.05$) of the total urine excretion	McHorse et al. (1975)
		20 = controls	- CYP2A6 lower metabolic activity in hepatitis patients	Case-control study
Hepatitis C virus (HCV) vs control	omeprazole (CYP2C19) and cortisol (CYP3A)	31 = HCV (9 with chronic hepatitis and	- mean omeprazole hydroxylation index in HCV patients were significantly higher compared with healthy subjects, with lower CYP2C19 activity	Smolders et al. (2017)
		22 with cirrhosis)	- mean clearance of cortisol decreased significantly ($p < 0.001$) in CLD patients	Case-control study
		30 = controls		
Chronic HCV treated with sofosbuvir	tacrolimus (CYP3A)	56-year-old male	- through concentration decreased after initiation of HCV treatment that required an increase of dosage	Kawaoka et al. (2016)
		74-year-old male		Case report
HCV treated with daclatasvir/asunaprevir	tacrolimus (CYP3A)	57-year-old man	- case 1: slight increase in trough blood concentration after the start of the combination therapy but no dose adjustment	Saab et al. (2016)
		63-year-old man	- case 2: through blood concentration decreased after the start of the combination therapy and dosage was increased	Case report
HCV before and after treatment	tacrolimus (CYP3A) and cyclosporine (CYP3A)	52	- statistically significant difference in daily dose adjusted per weight or serum levels of tacrolimus after achieving a sustained viral response	Raschzok et al. (2016)
			- no statistically significant difference in daily dose adjusted per weight or serum levels of cyclosporine after achieving a sustained viral response	Cohort study
HCV treated with directly acting antivirals	tacrolimus (CYP3A) and ^{13}C-methacetin (LiMAx test, CYP1A2)	21	- mean LiMAx increased from 344 ± 142 to 458 ± 170 μg/kg/h between the start of treatment and week 12 ($p < 0.001$) (value in healthy volunteers = 430 ± 86 μg/kg/h)	Ueda and Uemoto (2016)
			- tacrolimus C/D decreased over the same period ($p = 0.0017$)	Cohort study
HCV treated with daclatasvir/asunaprevir	tacrolimus (CYP3A)	10	- C/D ratio decreased from 3.95 ng/ml per mg to 2,975 ng/ml per mg after 2 weeks of administration	van den Berg et al. (2001)
				Cohort study
HCV	tacrolimus (CYP3A)	7 = HCV	- dose required to obtain therapeutic levels was comparable in the 2 groups during the first 3 weeks	Kugelmas et al. (2003)
		13 = transplanted for other indications	- dose requirement decreased sharply in HCV patients (20% of the value in controls)	Cohort study
			- dose requirement increased by more than 50% in 2 patients treated with IFN-α/ribavirin	
HCV treated with anti-HCV therapy	tacrolimus (CYP3A) and cyclosporine (CYP3A)	12 (7 cyclosporine and 5 tacrolimus) = responders	- cyclosporine and tacrolimus levels at baseline vs after HCV RNA negativation decreased significantly ($p = 0.018$ for cyclosporine and $p = 0.044$ for tacrolimus)	Ueda et al. (2015)
		18 (7 cyclosporine and 11 tacrolimus) = non-responders	- cyclosporine and tacrolimus levels in non-responders did not change between baseline and the end of anti-HCV therapy ($p = 0.24$ for cyclosporine and $p = 0.32$ for tacrolimus)	Cohort study
HCV treated with simeprevir	tacrolimus (CYP3A) and cyclosporine	2	- C/D ratio of calcineurin inhibitors were elevated in the first 2 weeks in both cases, but decreased thereafter, necessitating an increase in the dose	Morcos et al. (2013)
				Case report

be explained by the absence of correlation between its oral clearance and inflammatory markers in a cohort study, the lack of identical study conditions (doses, sample schedule, meals . . . etc.) between the two groups and the fact that HIV infection was well-controlled (Packageinserts; Le Tiec et al., 2005; Venuto et al., 2018). Indeed, caffeine metabolism was not altered in HIV-infected patient compared with healthy volunteers, but was decreased in AIDS patients (Lee et al., 1993; Jones et al., 2010). Moreover, atazanavir was administered with the booster ritonavir to decrease its clearance, and the effect of inflammation could have been minimized.

More recently, some studies have shown increased plasma concentration of CYPs substrates (mostly CYP3A) during SARS-CoV-2 infection, which may have led to believe that there was a CYPs downregulation due to inflammation (**Table 2D**) (Cojutti et al., 2020; Cranshaw and Harikumar, 2020; Gregoire et al., 2020;

TABLE 3C | Impact of HIV on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
AIDS patients vs control	clindamycin (CYP3A)	16 = AIDS	- clearance values normalized to subject body weight were 0.27 ± 0.06 L/h/kg for the healthy volunteers and 0.21 ± 0.06 L/h/kg for the AIDS patients (p = 0.014)	Breimer et al. (1975)
		16 = healthy volunteers	- ADR following administrations (same dose) were observed in eight patients with AIDS	Case-control study
HIV-infected patients vs control	midazolam (CYP3A), dextromethorphan (CYP2D6) and caffeine (CYP1A2)	17 = HIV-infected	- midazolam clearance was significantly lower in HIV-infected patient compared with healthy volunteers (CI95% = 0.68–0.92) and a significant relationship was found with TNF-α (r = –0.66, p = 0.008)	Imai et al. (2011)
		17 =	- urinary dextrometorphan MR was significantly higher in HIV-infected patients than in healthy volunteers (CI95% = 2.36–42.48) and a trend was observed for an association with the increase in TNF-α concentration (r = 0.49, p = 0.06)	Case-control study
		uninfected	- caffeine metabolism was no significantly different in HIV-infected subjects compared to non-smokers healthy volunteers (controlled for smoking status) (CI95% = 0.83–3.11)	
HIV-infected patients vs control	midazolam (CYP3A) and	30 = HIV-infected	- CYP3A4 activity in HIV infected patients was approximately 50% of the activity in healthy volunteers but it was mainly attributable to a lower intestinal CYP3A4 activity, while hepatic CYP3A was not different	Gatti et al. (1993)
	dextromethorphan (CYP2D6)	12 = healthy volunteers	- CYP2D6 activity was essentially comparable	Case-control study
HIV-positive patients	dextromethorphan (CYP2D6)	61	- 2 of the 59 patients with an NM genotype expressed a PM phenotype and 4 NM genotype patients were less extensive dextrometorphan metabolizers than any of the patients receiving medication known to inhibit CYP2D6	Jones et al. (2010)
				Cohort study
HIV-1 infected patients vs control	darunavir (CYP3A)	Unknown, information obtained from Summary of Product Characteristics (SmPC)	- exposure to darunavir was higher in HIV-1 infected patients	Jetter et al. (2010)
			- explained by the higher concentrations of α1-glycoprotrein in HIV-1 infected patients, resulting in higher darunavir binding to plasma AAG and, therefore, higher plasma concentrations	Case-control study
HIV-infected patients vs healthy volunteers	saquinavir (CYP3A)	33 = HIV-infected	- co-administration of ketoconazole increased saquinavir AUC by 190 and 69% in healthy volunteers and HIV-infected patients, respectively while co-administration of rifampicin decreased saquinavir area under the curve by 70 and 46%	European medicines agency
		12 and 14 = control		Case-control study
HIV-infected patients vs healthy controls	atazanavir and atazanavir with ritonavir (CYP3A)	Unknown, information obtained from SmPC	- mean AUC of atazanavir and atazanavir with ritonavir were 29′303 and 61′435 ng*h/mL respectively in healthy volunteers, vs. 22′262 and 53′761 ng*h/ml, respectively in HIV-infected patients	Grub et al. (2001)
				Case-control study
HIV-infected patients vs healthy controls	lopinavir with ritonavir (CYP3A)	Unknown, information obtained from SmPC	- no substantial differences observed between the two groups	Packageinserts
				Case-control study

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TABLE 3C | (*Continued*) Impact of HIV on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
HIV-infected patients vs healthy controls	atazanavir (CYP3A)	10 = HIV-infected	- mean atazanavir AUC in HIV-infected patients was 14′187 ng*h/ml compared with 33′097 ng*h/ml in healthy volunteers	Le Tiec et al. (2005)
		36 = healthy volunteers	- after 14 and 20 days of atazanavir in HIV patients and healthy volunteers, respectively, AUC were 46′073 and 57′039 ng*h/ml	Case-control study
Patients with different stage of HIV infection vs control	caffeine (CYP1A2)	29 = AIDS	- metabolic status was not change in HIV asymptomatic patients but changed in AIDS patients (with acute illnesses or stable)	Venuto et al. (2018)
		29 = AIDS-stable		Case-control study
		18 = HIV-infected 29 = control		
HIV infected patients	atazanavir (CYP3A)	107 = HIV-1 infected	- apparent oral clearance was not significantly correlated with inflammatory biomarkers	Lee et al. (1993)
				Cohort study

Marzolini et al., 2020; Schoergenhofer et al., 2020; Testa et al., 2020). Indeed, the plasma concentrations of some CYP3A substrates (lopinavir, darunavir and direct oral anticoagulants) were significantly increased in patients with SARS-CoV-2 infection (Cojutti et al., 2020; Gregoire et al., 2020; Schoergenhofer et al., 2020; Testa et al., 2020). CRP and IL-6 were also associated with lopinavir concentrations and a trend toward a return to baseline was observed after treatment with tocilizumab (Marzolini et al., 2020; Schoergenhofer et al., 2020). Indeed, lopinavir through level in patients with SARS-CoV-2 infection was twice as high as in HIV patients but concentrations decreased when tocilizumab was administered (Marzolini et al., 2020; Schoergenhofer et al., 2020). However, the impact of inflammation induced by SARS-CoV-2 infection on lopinavir through concentration may be also due to increased orosomucoid levels (Boffito et al., 2021; Stanke-Labesque et al., 2021). Lopinavir is a highly protein-bound drug and the misinterpretation of its overexposure during inflammation could be explained by the fact that total and not unbound concentration was considered (Boffito et al., 2021; Stanke-Labesque et al., 2021). Furthermore, a case report described clozapine toxicity and increased clozapine level from 0.57 to 0.73 mg/L during SARS-CoV-2 infection (Cranshaw and Harikumar, 2020). However, no correlation was found between CRP and hydroxychloroquine plasma concentrations (Marzolini et al., 2020).

Vaccination

Regarding vaccination (**Table 3**), several reports and studies assessed variations of PK/PD parameters of drugs after vaccination, but data remain contradictory. Of the 31 articles included, 28 were exclusively about influenza vaccination while two were about concomitant vaccinations including influenza (pneumococcus, tetanus and hepatitis A). Only one article did not evaluate the influenza vaccination but reported on the impact of *tuberculosis* vaccination (BCG). No significant difference of CYP activity between before or after vaccination was shown in several studies (Britton and Ruben, 1982; Fischer et al., 1982; Goldstein et al., 1982; Patriarca et al., 1983; Stults and Hashisaki, 1983; Stults and Hashisaki, 1983; Hayney and Muller, 2003). In particular, the impact of vaccination on anticoagulants effects has been well-studied but the majority of studies showed no variation of PT time or INR (Farrow and Nicholson, 1984; Kramer et al., 1984; Gomolin, 1986; Raj et al., 1995; Poli et al., 2002; Paliani et al., 2003; Iorio et al., 2006; Jackson et al., 2007; MacCallum et al., 2007; Casajuana et al., 2008). However, the occurrence of bleeding events a few days after vaccination, when the PT time was previously stable, has been described (Kramer et al., 1984; Weibert et al., 1986; Carroll and Carroll, 2009). Moreover, the case of a patient hospitalized because of serum CPK level of 93,000 U/L during treatment with cerivastatin and bezafibrate or the occurrence of tramadol toxicity has been reported (Plotkin et al., 2000; Pellegrino et al., 2013). The patient had been vaccinated 5 days earlier (Plotkin et al., 2000). Other studies, few in number, have found an effect of vaccination on the PK of CYP substrates (Renton et al., 1980; Kramer and McClain, 1981; Gray et al., 1983). However, no study has correlated the data with pro-inflammatory markers.

Organs Diseases

The influence of liver and kidney function on disposition of drugs excreted by the liver and kidney is widely recognized and used to derive dosing adaptations. However, there is now an increasing appreciation that kidney impairment can also reduce non-renal clearance and alter the bioavailability of drugs predominantly metabolized by the liver (Nolin, 2008). Indeed, uremic toxin has been implicated in transcriptional, translational and acute posttranslational modifications of CYP, and it has been recognized that inflammation is a common feature in end-stage renal disease (ESRD) patients (Nolin, 2008; Stenvinkel and Alvestrand, 2002). For example, CYP3A activity increased post-dialysis, meaning that it is the presence of uremic toxin that is responsible for CYP downregulation and not the underlying disease (Nolin et al., 2006). An inverse relationship between hepatic CYP3A activity was found in this study, but it did not prove causality (Nolin et al., 2006). It indicates that uremia can be used as a surrogate for dialyzable toxins that contribute to

TABLE 2D | Impact of SARS-CoV-2 on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
SARS-CoV-2 and treatment with tocilizumab	lopinavir/ritonavir (CYP3A) and hydroxychloroquine (CYP2D6)	41 = without tocilizumab, 51 = tocilizumab (35 before and 16 after)	- lopinavir concentrations positively correlated with CRP (r = 0.37, p < 0.001) and significantly lower after tocilizumab, - no correlation between CRP and hydroxychloroquine plasma concentration	Marzolini et al. (2020), Cohort study
SARS-CoV-2 vs. HIV-patients	lopinavir/ritonavir (CYP3A)	12	- lopinavir trough concentration in patients with SARS-CoV-2 infection were significantly higher than those usually observe in HIV-infected patients (18′000 vs. 5365 ng/ml)	Gregoire et al. (2020), Cohort study
SARS-CoV-2	clozapine (CYP1A2)	38-year-old-man	- symptoms of clozapine toxicity, - clozapine level increased by 0.57–0.73 mg/L and norclozapine increased by 0.22 mg/L to 0.31 mg/L after SARS-CoV-2 infection	Cranshaw and Harikumar (2020), Case report
SARS-CoV-2	lopinavir/ritonavir (CYP3A)	8	- through concentration associated with CRP level (r = 0.81, p = unknown), - through levels were 2-fold higher in patients with SARS-CoV-2 infection than HIV patients	Schoergenhofer et al. (2020), Cohort study
SARS-CoV-2	apixaban (CYP3A), rivaroxaban (CYP3A), edoxaban (CYP3A)	5 = apixaban, 3 = rivaroxaban, 3 = edoxaban	- alarming increase in DOAC plasma levels compared to pre-hospitalization levels, - possible role of concomitant drugs (CYP3A inhibitors) or disease-related organ dysfunctions	Testa et al. (2020), Cohort study
SARS-CoV-2 vs HIV-patients	darunavir (CYP3A)	30 = SARS-CoV-2 25 = HIV	- median CL/F was significantly lower in SARS-CoV-2 patients with IL-6 levels >18 pg/ml than <18 pg/ml or HIV patients (p < 0.0001), - increasing level of IL-6 affected concentration vs time simulated profile	Cojutti et al. (2020), Case-control study

alterations in CYP3A function (Nolin et al., 2006). Indeed, hemodialysis improved CYP3A activity with a 27% increase 2 h post-dialysis in uremic patients, suggesting that potential toxins responsible for this alteration were removed (Nolin et al., 2006). Authors suggested that this improvement occurred independently of transcriptional or translational modifications, contrary to what has been suggested previously (Nolin et al., 2006). However, as shown in **Table 4**, two studies found an association between the modification of CYP activity and inflammation in ESRD patients (Molanaei et al., 2012; Molanaei et al., 2018).

All studies in patients with liver disease described a decrease in CYP activity, compared to controls, as shown in **Table 5**. Indeed, several studies studied antipyrine, an old drug that is metabolized by multiple CYP (Branch et al., 1973; Farrell et al., 1979; Salmela et al., 1980; Teunissen et al., 1984; Schellens et al., 1989; Bauer et al., 1994; Grieco et al., 1998; Frye et al., 2006). They showed that CYP activity and antipyrine metabolism decreased only in severe disease compared to inactive cirrhosis, mild-moderate liver disease or healthy volunteers (Farrell et al., 1979; Bauer et al., 1994; Grieco et al., 1998). Moreover, chronic liver disease appeared to have a higher impact than an acute/reversible pathology (Branch et al., 1973). However, few studies have focused on a specific CYP substrate, and no studies found an association with inflammatory markers. One study demonstrated that CYP2C19, 2E1, 1A2 and 2D6 probe drugs concentrations were inversely correlated to the Child-Pugh score and another one demonstrated that phenacetin clearance decreased by 90% in patients with cirrhosis (Frye et al., 2006; Wang et al., 2010). Concerning CYP2C9, tolbutamide plasma levels increased by 10–20% and irbesartan AUC increased by 20–30% in cirrhotic patients (Ueda et al., 1963; Marino et al., 1998). The same results were found with CYP3A as diazepam clearance decreased in cirrhosis (Klotz et al., 1975). These variations may therefore be attributed to the loss of liver function due to tissue destruction. CYP metabolism appeared to be influenced by other organ's disease, such as clozapine serum levels that increased by 2-fold during chronic obstructive pulmonary disease (COPD) exacerbation and antipyrine clearance that was significantly lower in patient with COPD and antitrypsin deficiency than in healthy volunteers (Laybourn et al., 1986; Leung et al., 2014). In addition, one study showed that inflammatory markers were inversely correlated with CYP1A2 and CYP2C19 activity but not with CYP2D6 and CYP2E1 activity in patients with congestive heart failure (Frye et al., 2002).

Some studies conducted in critically ill patients (**Table 8**), showed that CYP1A2 and 3A metabolic activity were downregulated, and that it may be proportional to the severity and reversibility of the illness (Shelly et al., 1987; Toft et al., 1991; Kruger et al., 2009). For instance, theophylline clearance decreased by 10–66%, atorvastatin AUC increased by 15-fold, and clopidogrel active metabolite decreased by 48-fold, raising concerns about

TABLE 3 | Impact of vaccination on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Influenza vaccination	Erythromycin breath-tests (ERMBT) (CYP3A)	24 = healthy volunteers	- no significant difference between CYP3A4 activity before and 7 days after vaccination but the influenza antigen-specific production of IFN-γ by lymphocytes was highly correlated with the change in ERMBT (r = -0.614, p = 0.020) thus, IFN-γ downregulates the expression/activity of CYP3A4	Boffito et al. (2021) Non-random
Influenza vaccination	ERMBT (CYP3A)	15 = healthy volunteers	- significant inverse correlation between age and change in ERMBT (r = –0.624, p < 0.015) after vaccination	Stanke-Labesque et al. (2021) Non-random
Influenza vaccination	simvastatine (CYP3A)	68-year-old man	- hospitalized because of complaining of extreme weakness and diffuse muscle pain 5 days after influenza vaccine - 24 h after the vaccination, he began to complain of diffuse myalgia and symptoms worsened - serum CPK value at admission was of 93′000 U/L (70 U/L 2 weeks prior to admission)	Hayney and Muller (2003) Case report
Influenza vaccination	chloroxazone (CYP2E1)	10 = healthy volunteers	- no significant difference in the PK parameters before immunization and 7 and 21 days after vaccination	Stults and Hashisaki (1983) Non-random
Influenza vaccination vs controls	^{13}C-aminopyrine breath test (CYP2C19, 1A2 and 3A4)	12 = vaccinated	- significant reduction (22–74%, p < 0.001) in aminopyrine breath test 7 days after vaccination compared to controls	Fischer et al. (1982)
		10 = controls	- metabolic activity depression was not significant 2 days after vaccination but there was still a significant reduction 21 days after vaccination	Non-random
BCG vaccination (*tuberculosis*)	theophylline (CYP1A2)	9 = patients converted to positive Mantoux skin test	- the clearance and half-life were significantly decreased and increased, respectively (p < 0.02), in patients with positive Mantoux skin test, as compared to controls	Stults and Hashisaki (1983)
		3 = controls		Random
Influenza vaccination	theophylline (CYP1A2)	7=3 recovering from an acute exacerbation of COPD and 4 healthy volunteers	- plasmatic concentration before and after influenza vaccination significantly increased	Goldstein et al. (1982) Non-random
Influenza vaccination	theophylline (CYP1A2)	13	- no difference in the mean serum theophylline levels before influenza vaccination and 24h, 72h, 1 week and 2 weeks after vaccination	Britton and Ruben (1982) Non-random
Influenza vaccination	theophylline (CYP1A2)	7 (chronic bronchitis and chronic airflow obstruction thus and 5 men were smokers (CYP1A2 inductor))	- no difference between the clearance rate before and 24 h after vaccination (p = 0.778) - clearance 4–48 h after influenza vaccination was not significantly different (p = 0.789) - serum interferon was not detected in any of the seven subjects before or 8, 16, 24, 46 h and 7–10 days following vaccination	Patriarca et al. (1983) Non-random
Influenza vaccination	theophylline (CYP1A2)	16 (COPD)	- no difference in plasma concentration 24 h before or after vaccine injection	Jackson et al. (2007) Non-random

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TABLE 3 | (*Continued*) Impact of vaccination on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Influenza vaccination	theophylline (CYP1A2)	5	- no significant variations in the serum levels before and 24 h after vaccination	Farrow and Nicholson (1984) Non-random
Influenza vaccination	theophylline (CYP1A2) and chlordiazepoxide (CYP3A)	8 = theophylline	- an effect of vaccination has been shown on theophylline clearance at day 1 after vaccination ($p = 0.016$) but not at day 7	MacCallum et al. (2007)
		5 = chlordiazepoxide	- no effect on chlordiazepoxide metabolism - the effect seems to be greater when initial clearance is higher	Non-random
Influenza vaccination vs controls	theophylline (CYP1A2) and warfarin (CYP2C9)	152 = influenza vaccinated	- no ADR occurred in patients on theophylline in both groups and only one reaction in each group of patients who were taking warfarin	Raj et al. (1995)
		51 = unvaccinated		Case-control study
Influenza, pneumococcal, tetanus and hepatitis A vaccinations	warfarin (CYP2C9)	5′167	- not associated with INR value change	Gomolin (1986)
Influenza and pneumococcal vaccination vs. controls	warfarin (CYP2C9)	25 = placebo	- no statistically significant increments in mean British Corrected Ratios for prothrombin time 2, 7- or 21-days post injections	Cohort study Iorio et al. (2006)
		25 = influenza 19 = pneumococcal		Random
Influenza vaccination	warfarin (CYP2C9)	78	- no significant effect on anticoagulant control during the 10 days post-vaccination in the vast majority of individuals	Poli et al. (2002)
Influenza vaccination	warfarin (CYP2C9)	41	- no significant difference in the mean PT 3, 7 and 14 days after vaccination for the entire group and no patient developed any major or minor bleeding episodes	Cohort study Paliani et al. (2003)
Influenza vaccination vs controls	warfarin (CYP2C9)	7	- no difference in the mean PT one, three and 6 weeks after vaccination	Cohort study Casajuana et al. (2008)
Influenza vaccination	warfarin (CYP2C9)	104	- no difference in the mean PT-INR values and mean weekly dosage between group 1 (active vaccine at day 0 and placebo at day 42) and group 2 (placebo at day 0 and active vaccine at day 42)	Cohort study Kramer et al. (1984), Cross-over study
Influenza vaccination	warfarin (CYP2C9)	71 = vaccinated, 72 = controls	- no differences in the anticoagulation levels 3 months before and 3 months after the vaccination, - in the 34 vaccinated patients older than 70 years, a reduction of anticoagulation intensity was achieved in the 3 months after the vaccination and it was not the case in control group	Carroll and Carroll 2009), Case-control study
Influenza vaccination	warfarin (CYP2C9)	49 = patients, 45 = controls	- no difference in INR between patients and control groups before vaccination while 7–10 days after injection, INR significantly increased ($p < 0.00005$), - in patient group, INR increased significantly after vaccination ($p < 0.00001$)	Weibert et al. (1986), Case-control study

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TABLE 3 | (*Continued*) Impact of vaccination on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Influenza vaccination	225 acenocoumarol 4 warfarin (CYP2C9)	100 = intramuscular, 129 = subcutaneous	- INR decreased 24 h after intramuscular vaccination and increased in the subcutaneous group but the difference did not reach statistical significance	Plotkin et al. (2000), RCT
Influenza vaccination	warfarin (CYP2C9)	8	40% prolongation of PT (statistically significance unknown)	Pellegrino et al. (2013), Non-random
Influenza vaccination	warfarin (CYP2C9)	12 (healthy volunteers)	- no significant effect on warfarin metabolism was observed between influenza vaccination or saline injection	Pellegrino et al. (2013), Cross-over study
Influenza vaccination	warfarin (CYP2C9)	81-years-old man	- admitted with hematemesis and a 3-days history of melena and further investigations confirmed a bleeding gastric mucosa but no evidence of oesophagitis, gastritis, duodenitis or ulcer, - monthly PT had been stable and in the therapeutic ranges but the day of admission, PT was 36 s, - 10 days before admission, he received influenza vaccination. Warfarin was withheld and recovered uneventful	Pellegrino et al. (2013), Case report
Influenza vaccination	warfarin (CYP2C9)	64-years-old patient	- death from intracranial haemorrhage (INR = 15 at admission), - INR = 2 4.5 weeks before and all values over the previous 6 months were relatively stable, - vaccine 4.5 weeks before this fatal event	Kramer and McClain (1981), Case report
Influenza vaccination	warfarin (CYP2C9)	12	- small but significant increase in the PT ratio before and after vaccination, - maximal increase occurred on day 14 and represented a 7.6% increase over the baseline value	Gray et al. (1983), Non-random
Influenza vaccination	tramadol (CYP2B6 and 3A, bioactivated by CYP2D6)	85-years-old woman and a and 84-years-old man	- hallucinations and other neurologic symptoms six and 5 days after the administration of two different influenza vaccines	Renton et al. (1980), Case report
Influenza vaccination	carbamazepine (CYP1A2 and 2C9, bioactivated by CYP3A)	15-years-old woman	- vaccination 13 days before admission, but it was well tolerated, and no changes were made in her medication, - serum carbamazepine level was 27.5 μg/ml (ataxia and increasing lethargy) at admission and it decreased to 9.1 μg/ml 4 days after admission	Nolin (2008), Case report
Influenza vaccination	phenytoin (CYP2C9 and CYP2C19 substrates and induces CYP2C9, 2C19 and 3 A)	16	- no significant increase in mean serum concentration were observed on days 7 and 14 following the vaccination, - temporary increases of 46–170% mean serum concentration occurred in four subjects	Stenvinkel and Alvestrand (2002), Cohort study
Influenza vaccination	acetaminophen (CYP2E1), alprazolam (CYP3A), antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	24 (healthy volunteers 9 = acetaminophen, 7 = alprazolam, 8 = antipyrine)	- PK variables were no significantly different ($p > 0.05$) before and 7 and 21 days after vaccination	Nolin et al. (2006), Random

treatment efficacy (Toft et al., 1991; Kruger et al., 2009; Schoergenhofer et al., 2018). However, a systematic review reported that 20–65% of critically patients had an increased renal clearance, defined as a creatinine clearance greater than 130 ml/min/1.73 m^2 (Bilbao-Meseguer et al., 2018). This underscores the fact that inflammation has a different effect on drug clearance through the different mechanisms of drug elimination.

Diabetes

In diabetes (**Table 9**), CYP metabolism has been shown to be downregulated (Salmela et al., 1980; Pirttiaho et al., 1984).

TABLE 4 | Impact of renal diseases on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Severely impaired renal function vs normal	tolbutamide (CYP2C9)	11 = severe kidney impairment , 7 = normal	- Half-life was prolonged in severely impaired renal function patients (n = 11)	Molanaei et al. (2018), Case-control study
Haemodialyzed patients	alprazolam (CYP3A)	26	- ratio of unconjugated alprazolam to 4-hydroxyalprazolam was correlated with CRP levels (r = 0.49, p = 0.01) ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"Q0Jo8NiX","properties":{"formattedCitation":"(170)","plainCitation":"(170)","dontUpdate":true,"noteIndex":0},"citationItems":[{"id":1099,"uris":["http://zotero.org/users/2161612/items/8PPVMCBX"],"uri":["http://zotero.org/users/2161612/items/8PPVMCBX"],"itemData":{"id":1099,"type":"article-journal","abstract":"OBJECTIVE: To investigate the impact of persistent inflammation in hemodialysis (HD) patients on the pharmacokinetics of alprazolam, a cytochrome P450 (CYP) 3A4 substrate, and its metabolites and the role of HD in the impact of persistent inflammation in this clinical context.\nMETHODS: The study population comprised 26 HD patients (mean age 64 years, range 27-79 years; 19 men, 7 women) who were given 1 mg of alprazolam orally in the evening before the day of HD. Unconjugated and conjugated alprazolam and its 4-hydroxy and α-hydroxy metabolites were measured by liquid chromatography-mass spectrometry at 10, 34 (start of HD) and 38 (end of HD) h after intake. C-reactive protein (CRP) was measured weekly beginning 2 months before study initiation, and alpha 1-acid glycoprotein and 4β-hydroxycholesterol were measured at baseline. CYP3A4 activity was estimated as the ratio of unconjugated alprazolam to 4-hydroxyalprazolam between 10 and 34 h following alprazolam intake.\nRESULTS: After a single dose of alprazolam, plasma concentrations of unconjugated alprazolam and its metabolites decreased gradually, and unconjugated 4-hydroxyalprazolam was eliminated more rapidly than unconjugated alprazolam by HD. In contrast, the plasma concentrations of conjugated alprazolam and its conjugated metabolites increased during the 34 h following drug intake and the subsequent HD decreased their levels by almost 80%. The ratio of unconjugated alprazolam to 4-hydroxyalprazolam was correlated with CRP levels (r(s) = 0.49, P = 0.01). There was no significant correlation between CYP3A4 activity measured by alprazolam (4-hydroxylation) and alpha 1-acid glycoprotein or 4β-hydroxycholesterol. Conjugated alprazolam was also found in the plasma.\nCONCLUSIONS: The correlation between CYP3A4 activity (assessed by alprazolam 4-hydroxylation) and CRP level suggests that inflammation may downregulate CYP3A4 activity. If confirmed, this could have major implications for drug dosing in persistently inflamed patients.","container-title":"European Journal of Clinical Pharmacology","DOI":"10.1007/s00228-011-1163-8","ISSN":"1432-1041","issue":"5","journalAbbreviation":"Eur. J. Clin. Pharmacol.","language":"eng","note":"PMID: 22159869","page":"571-577","source":"PubMed","title":"Metabolism of alprazolam (a marker of CYP3A4) in hemodialysis patients with persistent inflammation","volume":"68","author":[{"family":"Molanaei","given":"Hadi"},{"family":"Stenvinkel","given":"Peter"},{"family":"Qureshi","given":"Abdul Rashid"},{"family":"Carrero","given":"Juan Jesús"},{"family":"Heimbürger","given":"Olof"},{"family":"Lindholm","given":"Bengt"},{"family":"Diczfalusy","given":"Ulf"},{"family":"Odar-Cederlöf","given":"Ingegerd"},{"family":"Bertilsson","given":"Leif"}],"issued":{"date-parts":[["2012",5]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}	Molanaei et al. (2012), Cohort study
Haemodialyzed patients	quinine (CYP3A)	44	- significant correlation between the ratio of quinine/3-OH-quinine and median CRP (r = 0.48, p = 0.001), orosomucoid (r = 0.44, p = 0.003) and IL-6 after 12 h after drug intake (r = 0.43, p = 0.004), - correlation is no longer significant for IL-6 and orosomucoid after adjustment for age, gender, diabetes mellitus, dialysis vintage, PTH, orosomucoid and medications and it remains borderline for CRP (r = 0.05)	Farrell et al. (1979), Cohort study
End stage renal disease (ESRD) vs. control	warfarin (CYP2C9)	7 = ESRD 6 = control	- 50% (p < 0.03) increase plasma warfarin S/R ratio relative to controls	Frye et al. (2006), Case-control study
Moderate and severe kidney impairment vs no/mild kidney impairment	warfarin (CYP2C9)	599 = no/mild 300 = moderate 81 = severe	- patients with moderate kidney impairment required 9.5% lower doses (p < 0.001) compared to controls, - patients with severe kidney impairment required 19.1% lower doses (p < 0.001) compared to controls, - reduced kidney function was associated with lower dose requirements independently of CYP2C9 and VKORC1 genotype and clinical factors	Grieco et al. (1998), Two cohort studies combined, Case-control study

TABLE 5 | Impact of liver diseases on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Mild to moderate hepatocellular changes or inactive cirrhosis and severe liver disease vs control	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	15 = mild-moderate hepatocellular damage, 13 = inactive cirrhosis, 22 = severe liver disease, 21 = controls	- mean value of hepatic CYP concentration did not differ between patients with mild to moderate hepatocellular changes (less than 50% hepatocytes morphologically abnormal) or inactive cirrhosis and controls and antipyrine half-life did not significantly differ between all groups, - CYP concentration was less in patients with severe liver disease (more than 50% hepatocytes morphologically abnormal or active cirrhosis) and, thus, antipyrine half-life was significantly lower ($p < 0.01$) compared to other groups	Bauer et al. (1994), Case-control study
Liver disease vs. control	caffeine (CYP1A2), mephenytoin (2C19), debrisoquin (2D6), and chlorzoxazone (2E1)	20 = liver disease	- significant decrease in metabolite production in patients with liver disease for CYP2C19 ($p < 0.001$), 2E1 ($p = 0.0081$), 1A2 ($p = 0.0054$) and 2D6 ($p = 0.0110$)	Salmela et al. (1980)
		20 = control	- each probe drug was significantly inversely related to the Pugh score	Case-control study
Chronic active hepatitis and cirrhosis vs. control	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	103 = controls, 101 = non-cirrhotic with liver metastases, 102 = chronic active hepatitis, 92 = confirmed cirrhosis, 120 = hepatocellular carcinoma and cirrhosis	- clearance was significantly impaired with respect to healthy volunteers, chronic hepatitis without fibrosis and non-cirrhotic patients with liver metastases, - mean clearance rate of the non-cirrhotic patients with liver metastasis was quite similar to that of patients with healthy livers, - cirrhotic patients with hepatocellular carcinoma also presented significantly impaired clearance compared with that of healthy volunteers and patients with liver metastasis, - elimination of antipyrine may very well be normal in patients with primary or metastatic liver disease, even when there is extensive tumour involvement	Branch et al. (1973), Case-control study
Cirrhotic patient and chronic hepatitis vs. control	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	6 = control, 6 = chronic active hepatitis, 5 = cirrhosis	- half-life and clearance were significantly higher and lower respectively in cirrhotic patients compared with healthy subjects, - no significant differences between hepatitis patients and healthy subjects	Schellens et al. (1989), Case-control study
Diabetics with fatty liver, fatty liver with inflammatory changes and with cirrhosis vs diabetics with normal liver	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	4 = control, 13 = fatty liver, 33 = fatty liver with inflammation, 6 = cirrhosis	- clearances decreased significantly in diabetics with fatty liver ($n = 13$, $p < 0.005$), in diabetics with fatty liver with inflammatory changes ($n = 33$, $p < 0.005$) and in diabetics with cirrhosis ($n = 6$, $p < 0.005$) as compared to diabetics with normal liver	Teunissen et al. (1984), Case-control study
Cirrhosis vs. normal	tolbutamide (2C9)	10 = cirrhotic patients, 7 = normal	- disappearance rate was reduced in five of ten cases, - half-life was prolonged to 7.8–11.2 h (4.4 h in normal group), - plasma levels after 24 h were 11.4–20.8% of the theoretical initial value (5.3% of the theoretical initial value in normal group)	Molanaei et al. (2018) Case-control study
Acute liver and chronic disease	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	14 = control, 38 = liver disease	- half-life was prolonged in patients with liver disease and those with chronic illness had greater increase than those with acute, reversible pathology	Wang et al. (2010), Case-control study
Various liver disease vs. controls	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4), hexobarbital (CYP2C19) and theophylline (CYP1A2)	24 = liver disease, 26 = controls	- clearance of antipyrine, hexobarbital and theophylline are lower than those found in the control subject	Liver disease = Ueda et al. (1963) , Controls = Marino et al. (1998), Case Control
Alcoholic cirrhosis vs. controls	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	23 = alcoholic liver cirrhosis, 17 = control	- clearance was significantly lower in patients with alcoholic cirrhosis as compared with healthy volunteers ($p < 0.001$), - the rates antipyrine formations metabolites were not reduced to the same extent	Klotz et al. (1975) Case-control study
Chronic hepatitis	mephenytoin (CYP2C9 and 2C19 and induces 2C9, 2C19 and 3 A)	35 = chronic hepatitis, 153 = controls	- mean metabolite excretion was significantly lower in patients with liver disease ($p < 0.005$)	Laybourn et al. (1986), Case-control study

(Continued on following page)

TABLE 5 | (*Continued*) Impact of liver diseases on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Liver disease	mephenytoin (CYP2C9 and 2C19 and induces 2C9, 2C19 and 3 A) and debrisoquin (CYP2D6)	18 = liver disease, 8 = controls	- urinary excretion of mephytoin's metabolite among patients with liver disease was significantly less than among the healthy controls (45% reduction), - the reduction in excretion of mephytoin depended on severity of the disease (28 and 62% decreases for patients with mild and moderate liver disease, respectively), - excretion of debrisoquin's metabolite was comparable between control and disease groups, as groups with mild or moderate disease	Frye et al. (2002), Case-control study
Cirrhotic vs. control	irbesartan (CYP2C9)	10 = hepatic impairment	- trend for moderate (20–30%) increase in AUC and Cmax values in the cirrhotic group compared with control group but the difference did not meet the predetermined criteria for clinical interest	Toft et al. (1991)
Hepatic impairment vs. control		10 = control	- no significant differences of mean half-life, Cmax, clearance and AUC, - patients with hepatic impairment had higher percentage of cumulative urinary extraction of unchanged irbesartan after multiple dose administration ($p < 0.05$)	Case-control study
Cirrhosis vs. control	meperidine (CYP2B6, 3A4 and 2C19)	10 = cirrhosis, 8 = control	- total plasma clearance was of 664 ± 293 ml/min in cirrhotic patients and of 1′316 ± 383 ml/min in healthy volunteers, - clearance was significantly reduced in cirrhosis patients ($p < 0.002$) ADDIN ZOTERO_ITEM CSL_CITATION {"citationID": "a2nlaknkd00","properties":{"formattedCitation": "(168)","plainCitation":"(168)","dontUpdate": true,"noteIndex":0},"citationItems":[{"id": 10553,"uris":["http://zotero.org/users/2161612/items/7HBDUYBB"],"uri":["http://zotero.org/users/2161612/items/7HBDUYBB"],"itemData": {"id":10553,"type":"article-journal","container-title":"Clinical Pharmacology and Therapeutics","DOI":"10.1002/cpt1974164667","ISSN":"0009-9236","issue": "4","journalAbbreviation":"Clin. Pharmacol. Ther.","language":"eng","note":"PMID: 4419525","page":"667-675","source": "PubMed","title":"The effect of cirrhosis on the disposition and elimination of meperidine in man","volume":"16","author":[{"family": "Klotz","given":"U."},{"family":"McHorse","given": "T. S."},{"family":"Wilkinson","given":"G. R."},{"family":"Schenker","given":"S."}],"issued": {"date-parts":[["1974",10]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}	Kruger et al. (2009), Case-control study
Cirrhosis vs. control	diazepam (CYP3A)	21 = liver disease (9 alcoholic liver cirrhosis, 8 acute viral hepatitis and 4 chronic active hepatitis), 33 = control	- half-life showed a more than 2-fold prolongation (105.6 ± 15.2 h vs. 46.4 ± 14.2 h, $p < 0.001$) in patients with cirrhosis compared with age-matched control groups, - a decrease in the total plasma clearance of the drug in cirrhosis ($p < 0.001$)	Shelly et al. (1987), Case-control study
Acute viral and chronic active hepatitis vs control			- patients with acute viral hepatitis had a half-life of 74.5 ± 27.5 h and those with active chronic hepatitis of 59.7 ± 23.0 h, as compared to a normal value in this age group of 32.7 ± 8.9 h ($p < 0.01$)	
Cirrhosis and chronic hepatitis B (CHB)	phenacetin (CYP1A2)	106 = cirrhosis, 41 = CHB, 82 = controls	- clearance decreased by 91.2% ($p < 0.01$) and 67.7% ($p < 0.005$) in the patients with cirrhosis ($n = 106$) and chronic hepatitis B ($n = 41$), respectively	Schoergenhofer et al. (2018), Case-control study

Indeed, antipyrine metabolism was decreased compared with controls in several studies (Salmela et al., 1980; Pirttiaho et al., 1984; Zysset and Wietholtz, 1988). One study using a cocktail approach showed that CYP2B6, CYP2C19 and CYP3A activity decreased, CYP1A2 and CYP2C9 activity increased, and CYP2D6 and CYP2E1 activity was unaffected in type II diabetes (T2D) (Gravel et al., 2019). However, conflicting results exist with tolbutamide and paracetamol half-lifes which were unchanged and increased respectively (Ueda et al., 1963; Adithan et al., 1988). Regarding CYP3A, one study found no impact on amlodipine or immunosuppressant metabolism while nisoldipine clearance was decreased (Wadhawan et al., 2000; Preston et al., 2001; Marques et al., 2002; Akhlaghi et al., 2012). The underlying mechanisms are associated with systemic inflammation and inflammatory

TABLE 6 | Impact of lung diseases on CYP activities.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
COPD exacerbation	clozapine (CYP1A2)	52-year-old woman	- symptoms of clozapine toxicity, - serum levels = 1400 ng/ml (References = 350–700 ng/ml)	Luong et al. (2016), Case reports
Chronic obstructive lung (COLD) and pulmonary disease caused by α1-antitrypsin (AAT) deficiency vs. control	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	35 = AAT, 25 = COLD, 31 = control	- clearance was not different in AAT and COLD patients ($p > 0.2$), - clearance significantly higher in healthy volunteers than in patients with COLD (18%, $p < 0.01$)	Bilbao-Meseguer et al. (2018), Case-control study

TABLE 7 | Impact of cardiac diseases on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Congestive heart failure	caffeine (CYP1A2), mephenytoin (2C19), dextromethorphan (2D6), chlorzoxazone (2E1)	16	- IL-6 levels were inversely correlated to CYP1A2 (r = -0.56, $p = 0.0235$) and CYP2C19 (r = -0.63, $p = 0.0094$) activities, - TNF-α was inversely correlated to CYP2C19 ($r = –0.61$, $p = 0.0118$) activity, - no significant relationship between IL-6 and TNF-α with CYP2D6 and 2E1 activities	Pirttiaho et al. (1984), Cohort study

cytokines. Indeed, it is well-established that chronic inflammation is involved in the pathophysiology of diabetes and the more complex condition of metabolic syndrome (Gravel et al., 2019). TNF-α can lead to the development of diabetes by affecting insulin action, and levels of inflammatory cytokines and markers are reported to be increased in diabetes patients (Darakjian et al., 2021). In a multivariate analysis, IFN-γ, IL-1β, IL-6 and TNF-α were associated with CYP activities, depending on the CYP isoenzyme (Gravel et al., 2019). However, type I (T1D) and type II diabetes did not appear to have the same impact on CYP metabolism (Dyer et al., 1994; Korrapati et al., 1995; Lucas et al., 1998; Zysset and Wietholtz, 1988; Matzke et al., 2000; Sotaniemi et al., 2002; Wang et al., 2003). The impact of inflammation may be different partly because of obesity, which is more common in T2D (Wang et al., 2003). Indeed, obese patients had a 40% increase in CYP2E1 activity (Lucas et al., 1998; Wang et al., 2003). CYP2E1 increased activity could also be attributed to hypo-insulinemia, as administration of insulin reverses this induction at the mRNA level (Lucas et al., 1998). Moreover, moderate controlled T1D had comparable CYP2E1 activity to healthy volunteers (Wang et al., 2003). This was confirmed in other studies that showed an unaffected metabolic clearance rate of antipyrine in well-controlled (by insulin) T1D (Zysset and Wietholtz, 1988; Sotaniemi et al., 2002). This could also be explained by insulin supplementation and the subsequent correction of ketones that leads to a return to baseline level for CYP2E1 expression (Wang et al., 2003). Indeed, ketones have been shown to be an important modulator of CYP2E1 by enhancing its protein expression and mRNA level (Wang et al., 2003). This has been confirmed with CYP1A2, where fluctuations in growth hormone levels, hyperketonemia and variation in glucose metabolic steady state and HbA1C levels may contribute to these changes (Bechtel et al., 1988; Korrapati et al., 1995; Matzke et al., 2000). The difference in classification criteria for T1D and type 2 diabetes may explain the inconsistent findings (Matzke et al., 2000). Further studies to discriminate between these two entities are needed (Zysset and Wietholtz, 1988).

Overall, CYP3A, 2C19 and 2B6 activity appear to be downregulated while CYP1A2 activity was increased and CYP2D6 activity was unchanged in diabetic patients (Bechtel et al., 1988; Urry et al., 2016; Gravel et al., 2019). Conflicting results remain regarding CYP2C9 and CYP2E1 (Ueda et al., 1963; Adithan et al., 1988; Lucas et al., 1998; Gravel et al., 2019).

Auto-Immune Diseases

Few studies observed the impact of auto-immune disease on CYP activities, such as psoriasis, systemic lupus erythematosus (SLE), Behçet's disease, rheumatoid arthritis (RA), Crohn's disease and celiac disease (**Table 10**). In contrast to what has been observed for CYP2D6 in other inflammatory states, two studies observed CYP2D6 downregulation in patient with SLE (Idle et al., 1978; Baer et al., 1986). However, these studies have some limitations, such as the presence of concomitant medications inhibiting the metabolism of CYP2D6 and the absence of adequate randomization (Baer et al., 1986). Even though RA is one of the most prevalent chronic inflammatory disease, only two case-control studies were found in the literature studying the impact of

TABLE 8 | Impact of critically ill patients on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Septicaemia with shock and respiratory failure and multiple organ failure (two or more organ dysfunction)	theophylline (CYP1A2) and ethylene-diamine (CYP3A)	6	- 10–66% reduction of theophylline clearance as compared to healthy volunteers. Half-life was 18.8 h compared to a normal value of 6 h, - 54% reduction of ethylenediamine clearance and half-life was 2.3 h, which is 5 times the normal value of 0.55 h	Zysset and Wietholtz (1988), Cohort study
Critically ill patients (ICU) with sepsis	atorvastatin (CYP3A)	12 = ICU with sepsis	- 18-fold higher Cmax ($p < 0.001$) and 15-fold higher AUC ($p < 0,01$)	Gravel et al. (2019)
vs control		5 = healthy volunteers		Case-control study
Critically ill patients	midazolam (CYP3A)	6	- CYP3A downregulation is proportional to the severity of the patient's illness and reversible, - normal values from other studies ADDIN ZOTERO_ITEM CSL_CITATION {"citationID": "a2lr6jrcbos","properties":{"formattedCitation": "(139)","plainCitation":"(139)","noteIndex": 0},"citationItems":[{"id":10589,"uris":["http://zotero.org/users/2161612/items/8UL6EWVY"],"uri":["http://zotero.org/users/2161612/items/8UL6EWVY"],"itemData":{"id": 10589,"type":"article-journal","container-title": "The Journal of Pharmacy and Pharmacology","DOI":"10.1111/j.2042-7158.1983.tb02960.x","ISSN":"0022-3573","issue":"6","journalAbbreviation":"J. Pharm. Pharmacol.","language":"eng","note":"PMID: 6135777","page":"378-382","source": "PubMed","title":"Comparative plasma pharmacokinetics of theophylline and ethylenediamine after the administration of aminophylline to man","volume":"35","author": [{"family":"Cotgreave","given":"I. A."},{"family": "Caldwell","given":"J."}],"issued":{"date-parts": [["1983",6]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} (139)	Preston et al. (2001), Case-control study
Multiply injured patients vs. healthy volunteers	mephenytoin (CYP2C19), chlorzoxazone (CYP2E1), dapsone (multiple CYP) and flurbiprofen (CYP2C9)	23 = multiple injured patients, 90 = control	- CYP2C19 and 2E1 activity significantly reduced in trauma patients as compared to healthy volunteers, - CYP2C9 and multiple CYP activities (dapsone) higher after injury as compared to healthy volunteers, - CYP2C19 and 2E1 activities correlated with MODS and MOF scores	Marques et al. (2002), Case-control study
Critically ill patients	clopidogrel (bioactivated by CYP2C19), pantoprazole (CYP2C19)	43 = clopidogrel, 16 = pantoprazole	- median ratio of clopidogrel active metabolite to clopidogrel concentration was 0.6 and this ratio was 48-fold higher ($p < 0.001$) in healthy volunteers, - 70% of critically ill patients were insufficiently treated with clopidogrel, - 5-fold increased pantoprazole half-life	Akhlaghi et al. (2012), Cohort study

RA on the PK and PD of verapamil and losartan, respectively (Mayo et al., 2000; Daneshtalab et al., 2006; Smolen et al., 2016). Verapamil is metabolized by CYP3A and 1A2 into norverapamil (Tracy et al., 1999). Verapamil and norverapamil metabolism has been shown to be reduced in patients with RA compared to healthy volunteers (Mayo et al., 2000). Verapamil was not more dromotropic or hypotensive in RA patients (Mayo et al., 2000). Inhibition of CYP2C9 was proportional to RA disease severity in another study, but this was not accompanied by reduced clinical response after losartan administration (Daneshtalab et al., 2006). Same results were found in patients with Behcet's disease. Indeed, one study observed downregulation of CYP2C9 in Behcet's patients (Goktaş et al., 2015). However, losartan's MR in nine patients with Behçet's disease taking colchicine were similar to those not taking colchicine (Goktaş et al., 2015). This may be because the drug had been taken for only 2 weeks (Goktaş et al., 2015).

In Crohn's disease, S-verapamil concentration was higher than R-verapamil while the opposite was found in normal conditions and higher plasma levels of propranolol were

TABLE 9 | Impact of diabetes on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Non-insulin dependent (NID) diabetic subjects with fatty liver vs. healthy subjects	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	21 = diabetes, 11 = control	- NID diabetic subjects with fatty liver have lowered hepatic drug metabolizing enzyme capacity as assessed per unit weight of liver tissue compared with healthy subjects ($p < 0.01$), - the relative clearance rate was significantly slower and the hepatic CYPs concentration lower than in non-diabetic controls ($p < 0.01$)	Wadhawan et al. (2000), Case-control study
Diabetes patients with normal liver	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	4 = diabetes, 13 = controls	clearance decrease significantly ($p < 0.005$) between diabetes patients with normal liver compared to controls	Teunissen et al. (1984), Case-control study
Type I and type II diabetes vs. controls	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	30 = diabetes (15 T1D and 15 T2D), 21 = controls (12 for T1D and 9 for T2D)	- half-life was reduced by 44% compared to the controls ($p = 0.002$), whereas the resulting plasma clearance did not differ between controls and type I diabetics (T1D), - Type II diabetics (T2D) showed a 31% increase in plasma half-life ($p = 0.05$) and they had a significant decrease in corresponding clearance ($p = 0.02$)	Darakjian et al. (2021), Case-control study
Type I and type II diabetes vs. controls	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4), caffeine (CYP1A2) and dextromethorphan (CYP2D6)	15 = T1D, 16 = T2D, 16 = controls	- metabolism was significantly higher in T1D patients than in the patients with T2D and in healthy volunteers, - no change in metabolism between T2D and controls, - CYP1A2 activity was 34 and 42% higher in patients with T1D compared with controls and patients with T2D respectively but these changes did not reach the statistical significance ($p = 0.11$), - no change between groups concerning the CYP2D6 phenotype distribution	Matzke et al. (2000), Case-control study
Type II diabetes vs control	caffeine (CYP1A2) bupropion (CYP2B6), tolbutamide (CYP2C9), omeprazole (CYP2C19), dextrometorphan (CYP2D6), chlorzoxazone (CYP2E1) and CYP3A (midazolam)	38 = T2D, 35 = control	CYP2B6, CYP2C19 and CYP3A activities were decreased by about 45% ($p = 0.01$), 46% ($p = 0.001$) and 38% ($p < 0.0001$) respectively in T2D patients and multivariate models showed that IFN-γ and TNF-α, pro-inflammatory cytokines, partly explain these variations, - CYP1A2 and CYP2C9 metabolic activity were increased in T2D patients ($p = 0.008$ and $p = 0.0008$, respectively) at first sight but this is no longer significant when they have been adjusted for age and gender ($p = 0.07$ and $p = 0.05$, respectively), - CYP2D6 and CYP2E1 activities were not affected by diabetic status ($p = 0.75$ and $p = 0.78$, respectively), - phenotypes were extrapolated from genotypes because patients did not take other co-medications and there is no interaction between genotype/phenotype classification and diabetic status	Lucas et al. (1998), Case-control study
Type II diabetes vs. control	caffeine (CYP1A2)	57 = T2D, 146 = control	- metabolic activity of CYP1A2 was significantly increased in T2D patients compared to control ($p = 0.010$), - but when the 19 diabetic patients who are under insulin injection were removed, the difference was no longer significant ($p = 0.121$)	Dyer et al. (1994), Case-control study
Insulin dependent (ID) diabetes patients vs. control	caffeine (CYP1A2) and debrisoquin (CYP2D6)	28 = ID diabetes patients, 22 = healthy volunteers	- no significant differences for CYP2D6 activity and a significant increase in CYP1A2 activity in diabetes patients ($p < 0.0001$)	Wang et al. (2003), Case-control study
T1D and T2D vs. control	caffeine (CYP1A2)	10 = T1D; 8 = controls, 9 = T2D; 9 = controls	the apparent volume of distribution, apparent clearance, half-life, and peak concentrations of caffeine did not differ between both type of diabetes and controls	Sotaniemi et al. (2002), Case-control study
Diabetic patients vs. controls	tolbutamide (CYP2C9)	10 = diabetic patients, 7 = control	half-life in diabetic patients revealed no significant difference with normal subjects ADDIN ZOTERO_ITEM CSL_CITATION {"citationID": "yU0UBeFO","properties":{"formattedCitation":"(115)","plainCitation": "(115)","noteIndex":0},"citationItems":[{"id":10235,"uris":["http://zotero.org/users/2161612/items/ELGVD5C6"],"uri":["http://zotero.org/users/2161612/items/ELGVD5C6"],"itemData":{"id": 10235,"type":"article-journal","container-title":"Diabetes","DOI": "10.2337/diab.12.5.414","ISSN":"0012-1797","journalAbbreviation": "Diabetes","language":"eng","note":"PMID: 14067739","page":"414-419","source":"PubMed","title":"DISAPPEARANCE RATE OF TOLBUTAMIDE IN NORMAL SUBJECTS AND IN DIABETES MELLITUS, LIVER CIRRHOSIS, AND RENAL DISEASE","volume": "12","author":[{"family":"Ueda","given":"H."},{"family": "Sakurai","given":"T."},{"family":"Ota","given":"M."},{"family": "Nakajima","given":"A."},{"family":"Kamii","given":"K."},{"family": "Maezawa","given":"H."}],"issued":{"date-parts": [["1963",10]]}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} (115)	Molanaei et al. (2018), Case-control study
Diabetes mellitus vs. controls	paracetamol (CYP2E1)	19 = diabetes mellitus, 10 = healthy volunteers	- half-life was significantly increased ($p < 0.001$) with a corresponding decrease in clearance ($p < 0.001$) when compared with healthy volunteers, - clearance in patients with T2D was significantly decreased compared to T1D patients ($p < 0.01$) but it was not the case for its half-life, - the distribution volume was increased in patients with T1D compared to patients with T2D ($p > 0.05$)	Korrapati et al. (1995), Case-control study
Type II diabetes vs control	amlodipine (CYP3A)	18 = T2D, 20 = control	-no significant difference in AUC in hypertensive patients with and without T2D	Bechtel et al. (1988), Case-control study
Type II diabetes vs control	nisoldipine (CYP3A) and lidocaine (CYP3A)	17 = T2D, 10 = control	- the apparent clearances of both nisoldipine enantiomers in the hypertensive patients with T2D are significantly lower than in hypertensive control patients ($p < 0.05$), - higher ratio of plasma lidocaine/MEGX concentration for diabetic group than in control group ($p < 0.05$), - means that CYP3A4 activities were decreased in the diabetic groups, - significant correlations were found ($p < 0.05$) between the MR of lidocaine and the apparent clearance of nisoldipine enantiomers obtained for both groups	Urry et al. (2016), Case-control study
Diabetes vs. control	CyA (CYP3A)	7 = diabetes, 10 = control	-No difference was found in daily dose needed between both groups ($p = 0.55$) but metabolite-parent concentration ratios for all metabolites except one (AM4N, $p = 0.93$) were significantly lower in diabetic patients ($0.0001 < p$-value < 0.04)	Idle et al. (1978), Case-control study

(Continued on following page)

TABLE 9 | (*Continued*) Impact of diabetes on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Diabetes vs. control	CyA (CYP3A)	8 = diabetes, 9 = control	AUC adjusted with dosage was significantly lower in diabetic group (p = 0.03) ADDIN ZOTERO_ITEM CSL_CITATION {"citationID": "atdeho0nge","properties":{"formattedCitation": "(194)","plainCitation":"(194)","dontUpdate":true,"noteIndex": 0},"citationItems":[{"id":11162,"uris":["http://zotero.org/users/2161612/items/KYQT5CPG"],"uri":["http://zotero.org/users/2161612/items/KYQT5CPG"],"itemData":{"id":11162,"type":"article-journal","abstract":"BACKGROUND AND OBJECTIVES: Long-term diabetes mellitus may affect the absorption, distribution and metabolism of immunosuppressive agents used after organ transplantation. The aims of this study were to characterize ciclosporin pharmacokinetics in blood and plasma and to compare the ciclosporin unbound concentration and the blood : plasma concentration (B : P) ratio in diabetic kidney transplant recipients.\nPATIENTS AND METHODS: Ciclosporin 12-hour steady-state pharmacokinetics were studied in eight diabetic and nine nondiabetic patients. Ciclosporin concentrations in whole blood and in plasma were measured using liquid chromatography-tandem mass spectrometry, and the ciclosporin fraction unbound (f(u)) was determined by an equilibrium dialysis method utilizing [(3)H]ciclosporin as a tracer. Oral absorption of paracetamol (acetaminophen) was used as a marker for gastric emptying.\nRESULTS: In diabetic patients, the time to the peak blood ciclosporin concentration at steady state (t(max)(,ss)) was prolonged (128 minutes vs 93 minutes in nondiabetic patients, p < 0.01) and, on average, the paracetamol t(max) was prolonged by 30 minutes. The whole-blood dose-normalized area under the concentration-time curve from 0 to 12 hours (AUC(12)) was marginally lower in diabetic patients (p = 0.09) and the plasma AUC(12) was significantly lower (p = 0.03). The ciclosporin f(u) was numerically higher in diabetic patients (1.20 +/- 0.65% vs 0.72 +/- 0.28% in nondiabetic patients, p = 0.066); however, the unbound concentration values were essentially similar in the two groups (0.58 +/- 0.76 microg/L in diabetic patients and 0.52 +/- 0.48 microg/L in nondiabetic patients; p = 0.59). No difference was observed in the ciclosporin B : P ratio between the two groups.\nCONCLUSION: This study indicates that diabetes delays ciclosporin absorption, reduces ciclosporin exposure and increases the ciclosporin f(u) but not the pharmacologically active unbound concentration.","container-title":"Clinical Pharmacokinetics","DOI": "10.2165/00003088-200847110-00004","ISSN":"0312-5963","issue":"11","journalAbbreviation":"Clin Pharmacokinet","language":"eng","note":"PMID: 18840028","page": "733-742","source":"PubMed","title":"Blood and plasma pharmacokinetics of ciclosporin in diabetic kidney transplant recipients","volume":"47","author":[{"family":"Mendonza","given": "Anisha E."},{"family":"Gohh","given":"Reginald Y."},{"family": "Akhlaghi","given":"Fatemeh"}],"issued":{"date-parts": [["2008"]]}}},"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}	Baer et al. (1986), Case-control study
Diabetes vs. control	CyA (CYP3A)	36 = diabetes, 67 = control	- no difference was found concerning dose and through levels	Smolen et al. (2016), Case-control study
Type I and II diabetes vs control	chlorzoxazone (CYP2E1)	7 = T1D, 15 = T2D, 42 = controls	- no difference was found concerning CYP2E1 activity between groups	Mayo et al. (2000), Case-control study
Type II diabetes vs. control	quinine (CYP3A)	12 = T2D, 10 = controls	- PK parameters were comparable in the two groups (p > 0.02)	Daneshtalab et al. (2006), Case control study
Type I and II diabetes vs control	chlorzoxazone (CYP2E1)	14 = T1D, 8 = T2D, 10 = controls	- 2-fold increase in the oral clearance (p < 0.05) in T2D patients compared with T1D and controls, - no difference in oral clearance between T1D and controls	Tracy et al. (1999), Case-control study
Type I and type II diabetes	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	139 = T1D (120 = controls), 99 = T2D (70 = controls)	- clearance decreased in T2D patients as compared to controls, - metabolism is rapid in T1D patients	Goktaş et al. (2015), Case-control study
Type 1 diabetes vs controls	theophylline (CYP1A2)	8 = T1D, 8 = controls	- mean plasma clearance and elimination half-life did not differ significantly between the 2 groups	Sanaee et al. (2011), Case-control study
Gestational diabetes vs. pregnant women	metoprolol (CYP2D6)	10 = diabetes, 13 = control	- PK of the metoprolol isomers in the pregnant women and in gestational diabetes groups did not differ significantly, except for the R-metoprolol half-life (p < 0.05)	Schneider et al. (1976), Case-control study
Gestational diabetes vs. pregnant women	lidocaine (CYP3A)	6 = diabetes, 10 = control	- the ratios of lidocaine and its metabolite MEGX concentrations (lidocaine/MEGX ratio) at 15 and 30 min were significantly higher in the pregnant women with gestational diabetes mellitus compared to the normal pregnant women (58.34 vs. 23.21 at 15 min and 37.52 vs. 15.80 at 30 in, p < 0.05)	Lebwohl et al. (2018), Case-control study

found in Crohn's with reduced metabolic activities of CYP1A2, 2D6 and 2C19 (Schneider et al., 1976; Sanaee et al., 2011). Furthermore, there were no difference between healthy controls and Crohn's disease patients in remission, implying that CYP downregulation is proportional to disease severity and that recovery resulted in a return to baseline metabolic activity (Sanaee et al., 2011). Norverapamil goes through the same process and it is expected that the enantiomers ratio of norverapamil to verapamil remains unchanged (Sanaee et al., 2011).

TABLE 10 | Impact of autoimmune diseases on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Psoriasis vs healthy volunteers	venlafaxine (CYP2D6)	13 = psoriasis, 11 = control	- PK of the enantiomers and of its metabolites were not altered as compared to control	Lang et al. (1996) Case-control study
Systemic lupus erythematosus (SLE) vs. healthy controls	debrisoquin (CYP2D6)	42 = SLE, 147 = control	- In patients with SLE, there is an inhibition in the metabolism of debrisoquin compared to controls because there is significantly more PM patients in patients group ($p < 0.04$)	Tidball (2005), Case-control study
Proctitis vs healthy volunteers	/	11	- patients who suffered from proctitis showed a lower CYP2E1 and 3A4 gene expression in rectal mucosa with severe inflammation compared to normal mucosa ($p < 0.05$), - no significant difference for CYP3A5 ($p = 0.08$)	Baigrie et al. (1992), Cohort study
Behçet's disease vs. healthy subjects	losartan (CYP2C9)	52 = Behçet's disease, 73 = control	- the MR (losartan/E-3174) significantly increase ($p = 0.002$) compare to controls already included who genetic variants and losartan oxidation were already known, - in patients with the wild type CYP2C9 genotype (*1/*1), the MR significantly increased in patients with Behçet's disease compared to controls ($p = 0.006$) but there is no significant differences found for other CYP2C9 genotype	Bergin et al. (2011), Case-control study
Rheumatoid arthritis (RA) vs. healthy volunteers	verapamil (CYP3A4, 1A2, 2C8, 2C9 and 2C18)	8 = RA, 8 = controls	- less metabolized and bound to protein in patients with RA compared to controls, - AUC of verapamil and norverapamil were significantly higher in patients with RA as compared to controls thus, there is no changes in metabolite to parent drug ratio	Haas et al. (2003), Case-control study
Active and controlled rheumatoid arthritis vs healthy subjects	losartan (CYP2C9)	14 = active RA, 12 = controlled RA, 12 = controls	- PK not significantly altered but AUC of its pharmacologically active metabolite was significantly decreased , - MR exhibited a significant correlation with disease severity ($r = -0,35$, $p < 0.05$)	Lenoir et al. (2020), Case-control study
Rheumatoid arthritis	/	49 = RA	- cytokines such as TNF-α, IL-1β and IL-17 increase the CYP7B activity in synovial tissue, - TGF-β down-regulate the CYP7B activity and it results in enhanced formation of 7α-OH-DHEA in the arthritic joint, which may contribute to the maintenance of the inflammation and, thus, the chronicity of the inflammation response	Mostowik et al. (2015), Cohort study
active Crohn's disease (CD), Crohn's disease in remission and healthy subjects	verapamil (CYP3A4, 1A2, 2C8, 2C9 and 2C18)	22 = CD remission, 14 = CD active, 9 = controls	- plasma S-verapamil concentration in patients with active CD was significantly higher than in both healthy controls and patients in CD remission ($p < 0.001$) but not between healthy controls and Crohn's disease remission, - same tendency was seen for R-verapamil but there is no statistical significance, - as in RA patients, the ratio AUC of both S and R norverapamil over their corresponding verapamil enantiomers were not significantly different among the 3 groups of subjects, - there was no higher PD response in patients due to higher verapamil level	Bernlochner et al. (2010), Case-control study
Crohn's disease vs. control	propranolol (CYP2D6)	10 = Crohn's disease, 12 = healthy subjects	- levels were significantly higher in the 10 patients with Crohn's disease than those of the controls ($p < 0.05$)	Harvey and Morgan (2014), Case-control study
Celiac disease	/	9	- reduction in the intestinal content of CYP3A in patients with celiac disease before treatment with a gluten-free diet and increase in intestinal CYP3A protein after the diet	Kacevska et al. (2008), Cohort study

Celiac disease is an autoimmune disease that is triggered by an immune response to gluten and may result in increased morbidity or mortality (Lebwohl et al., 2018). The reduction in intestinal CYP3A content during celiac disease and its increase after a gluten-free diet indicate that local inflammation reduced CYP3A activity but that it returns to baseline with disease improvement (Lang et al., 1996).

TABLE 11 | Impact of surgery on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Surgery	clozapine (CYP1A2)	49-year-old man	- clozapine and norclozapine levels were 1130 ng/dl and 297 ng/dl, respectively (ratio 3.8: 1), 4 days after surgery. On day 2, dosage was reduced due to persistent sedation	Luong et al. (2016), Case reports
(a) Surgery abdominal aortic bypass graft colon resection peripheral vascular bypass graft	/ carbon-14 [^{14}C] ERMBT (CYP3A)	16 (5 a, 6 b and 5 c)	- ERMBT results significantly declined in all groups compared with before surgery - a trend toward difference in ERMBT results between surgery but didn't reach statistical significance (p = 0.06) - the nadir ERMBT result was significantly and negatively correlated (r = -0.541, p = 0.03) with peak IL-6 concentration - test results were significantly different if patients IL-6 peak concentration was IL-6 > 100 pg/ml or <100 pg/ml (35.5 vs. 74.7%, p < 0.001)	Chen et al. (1994) Cohort study
Hip surgery	caffeine (CYP1A2), bupropion (CYP2B6), flurbiprofen (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6) and midazolam (CYP3A)	30	- CYP2C19 and 3A MR decreased by 57% (p = 0.0002) and 61% ($p \leq 0.0001$) respectively with the nadir at D3, - CYP1A2 MR decreased by 53% ($p \leq 0.0001$) with the nadir at D1, - CYP2B6 and 2C9 MR increased by 120% (p < 0.0001) and 79% (p = 0.0018), respectively and peaked at d1, - No change in CYP2D6 MR	Rivory et al. (2002), Cohort study
percutaneous coronary intervention	clopidogrel (bioactivated by CYP2C19)	50	- prolonged post-angioplasty increase is associated with lower platelets' response to clopidogrel	Alexandre et al. (2007), Cohort study
percutaneous coronary intervention	clopidogrel (bioactivated by CYP2C19)	1′223	- platelet aggregation was significantly higher in patients with elevated CRP levels compared to patients with normal CRP levels (p < 0.001)	Charles et al. (2006), Cohort study

Surgery

The impact of surgery on concomitant treatment and analgesia management has been assessed in several studies (**Table 11**). Surgery is associated with an inflammatory response due to muscle or tissue injury to induce repair, regeneration and growth and so inflammatory markers increase after surgery, but not equally (Tidball, 2005; Stavropoulou et al., 2018). IL-1β was only detected during the early perioperative period and for a very short time (Baigrie et al., 1992). IL-6 plasma level peaked 4–48 h after surgery and declined drastically by 48–72 h in all patients without any postoperative complication (Baigrie et al., 1992). CRP level rose more slowly postoperatively compared with the cytokine levels (IL-6, TNF-α and IL-1β) (Bergin et al., 2011). Acute inflammation after elective surgery was associated with a significant decrease in CYP3A metabolic activity (Haas et al., 2003). A recent study with a cocktail approach has concluded that there is an isoform specific impact of inflammation on CYP activities (Lenoir et al., 2020). Indeed, this study showed that CYP1A2, CYP2C19 and CYP3A activities decreased significantly by 53, 57 and 61%, whereas CYP2B6 and CYP2C9 activities increased significantly by 120 and 79% (Lenoir et al., 2020). However, surgery did not significantly impact CYP2D6 activity (Lenoir et al., 2020). These findings were confirmed by a case report that showed a toxic increase in clozapine levels 4 days after surgery and by authors who further showed that clopidogrel efficacy was reduced in patients undergoing percutaneous coronary intervention, because clopidogrel must be bioactivated by CYP2C19 to be effective (Bernlochner et al., 2010; Leung et al., 2014; Mostowik et al., 2015).

Cancer

Inflammation is linked to all stages of cancer (risk of development, initiation, invasion, metastasis and mortality) as highlighted in **Table 12** (Harvey and Morgan, 2014). Certain immune-mediated diseases have been associated with cancer such as inflammatory bowel disease (IBD), chronic infection by Helicobacter pylori and chronic psoriasis associated with an increased risk of colorectal, gastric and skin cancer, respectively (Harvey and Morgan, 2014). The first pro-cancer immune signals are via tumor cells that successively produce cytokines and act to increase transcription factors, induce epigenetic changes and initiate angiogenesis (Harvey and Morgan, 2014). Cytokines are involved from neoplastic transformation of cells to tumor progression and metastasis, and are thus involved in several cellular events leading to cancer (Kacevska et al., 2008). These signals and others induced to respond to cancer are opposed by antigen-presentating cell-mediated anticancer immune responses (Harvey and Morgan, 2014). Moreover, the greater the antitumoral response is, the more the cancer outcome is improved whereas some T-cells subsets are associated with tumor promotion (Harvey and Morgan, 2014). Some cytokines have tumor-promoting, antitumor effects or both (Kacevska et al., 2008). Some cytokines could be produced by the tumor itself

TABLE 12 | Impact of cancer on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Liver metastasis before cytostatic treatment vs. healthy controls	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	12 = liver metastasis, 12 = controls	- no significant difference between patients with liver metastases before cytostatic treatment and controls	Williams et al. (2000), Case-control study
Bone marrow transplantation for haematological malignancies (radiation and chemotherapy)	CyA (CYP3A)	6	- concentration peak value occurred 15.8 days after bone marrow transplantation and it's corresponded to a 3- or 4-fold increase relative to the steady state day ($p > 0.015$), - CyA concentration peak and IL-6 peak levels are interdependent because there was a correlation between these two parameters ($r = 0.794$, $p = 0.03$)	Burns et al. (2014), Cohort study
Cancer	ERMBT (CYP3A)	40	- patients with CRP >10 mg/L had an average 30% reduction in CYP3A4 metabolic activity ($p = 0.0062$), - 1/Tmax values were negatively correlated with both CRP ($r = –0.64$, $p < 0.00001$) and α-glycoprotein ($r = -0.45$, $p < 0.005$), - 3 patients were treated by a CYP3A4 inhibitor while 4 patients were on long-term treatment with dexamethasone (inducer) but correlation with CRP remained significant ($r = –0.55$, $p = 0.002$) after removal of these patients	Helsby et al. (2008), Cohort study
Advanced cancer patients with normal liver function	midazolam and docetaxel (CYP3A)	56	- high midazolam concentration and free docetaxel AUC were associated with sever neutropenia (and conversion to febrile neutropenia), - high midazolam concentration was correlated with elevated ferritin level ($r = 0.32$, $p = 0.02$) (indicator of an inflammatory state), - according to authors, inflammation favors a reduction in CYP3A activity and thus, could lead to an overexposure to its substrates	Yasu et al. (2017), Cohort study
Advanced cancer patients who were suitable for palliative chemotherapy	docetaxel (CYP3A)	68	- occurrence of grade 3/4 non-haematological toxicities were not associated with high docetaxel exposure but with baseline concentrations of AAGP ($p = 0.03$) and CRP ($p = 0.05$), - results from correlation analysis between inflammation markers and docetaxel clearance were not given, as the results from EBT	Mafuru et al. (2019), Non-randomized clinical trial
Cancer patients vs healthy subjects	omeprazole (CYP2C19)	16 = cancer, 77 = controls	CYP2C19 activity differed significantly ($p < 0.0001$) in the EM cancer patients compared of the References population with EM genotype	Piscitelli et al. (1998), Case-control study
Multiple myeloma	proguanil (CYP2C19)	25	- significant discordance between the CYP2C19 activity predicted by genotype and the measured phenotype ($p < 0.0001$), - no significant difference in CRP and IL-6 concentrations between discordant and concordant subjects ($p = 0.072$ and $p = 0.694$, respectively)	Elkahwaji et al. (1999), Cohort study
Advanced cancer	omeprazole (CYP2C19)	31	- comparison of the predicted phenotype from genotype and the measured MR of CYP2C19 found a statistically discordance ($p < 0.0005$), - of the 30 cancer patients with genotypic EM status, 11 were CYP2C19 PM, - no significant correlation between the levels of any individual cytokine (CRP, IL-1β, Il-1α, IL-6, TNF-α, TGF-β and CRP) and CYP2C19 metabolic activity	Israel et al. (1993), Cohort study
Hematopoietic cell transplantation	voriconazole (CYP3A4 and CYP2C19)	67	- CRP levels were significantly correlated ($r = 0.22$, $p < 0.001$), - higher voriconazole trough concentration >1.0 ug/ml was observed in higher CRP level >4 mg/dl	Jonkman et al. (1989), Cohort study

(Continued on following page)

TABLE 12 | (*Continued*) Impact of cancer on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Hematologic patients	voriconazole (CYP3A4 and CYP2C19)	113	- concentration was significantly correlated with IL-18 in acute myeloid ($r = 0.456$, $p < 0.0001$), acute lymphoblastic ($r = 0.317$, $p = 0.019$), and chronic myeloid leukaemia ($r = 0.737$, $p = 0.04$), - concentration and TGF-β1 were correlated ($r = 0.436$, $p < 0.001$) in acute myeloid leukaemia patients only, - according to authors, IL-6 level could partially predict the voriconazole trough concentration because these two factors were weakly inversely correlated in hematologic patients regardless of underlying disease	Williams et al. (1987), Cohort study
Hepatocellular carcinoma	phenacetin (CYP1A2)	148 = carcinoma, 82 = controls	- clearance did not significantly differ between the healthy participants and patients with hepatocellular carcinoma	Schoergenhofer et al. (2018), Case-control study

(Kacevska et al., 2008). Inflammation has therefore a pivotal role in cancer and the proliferation of malignant cells by a dynamic equilibrium in the tumor environment (Harvey and Morgan, 2014). Cytokines present in the tumor environment are also launched in the systemic circulation and have general effects on the function of distant organs such as the liver (Kacevska et al., 2008). Inflammatory markers levels are dependent on tumor types, but high level of CRP, IL-6, IL-1β have been associated with poor prognosis (Kacevska et al., 2008). Some results suggest that high IL-6 is associated with decreased CYP3A metabolic activity but can also nonspecifically downregulate CYP-dependent drug metabolism (Chen et al., 1994). CRP and α-glycoprotein were also negatively correlated with CYP3A activity and cancer patients with significant acute-phase response may have reduced CYP3A drug metabolism, which may have implications for the safety and efficacy of chemotherapy (Rivory et al., 2002; Charles et al., 2006; Alexandre et al., 2007). Inflammatory status and lymphocyte count should thus be included in the evaluation of the benefit/risk ratio before the initiation of a cytotoxic chemotherapy (Alexandre et al., 2007). Concerning CYP2C19, studies showed that CYP2C19 activity was not solely predicted by the genotype in cancer patients (Williams et al., 2000; Helsby et al., 2008; Burns et al., 2014). Indeed, CYP2C19 activity was reduced in cancer patients, with a discordance between the measured phenotype and the predicted phenotype from the genotype. However, no significant correlation was found between CYP2C19 activity and the levels of cytokine, whereas this was the case for voriconazole through concentration (Helsby et al., 2008; Burns et al., 2014; Yasu et al., 2017; Mafuru et al., 2019). The mechanism behind the decrease of CYP2C19 activity observed in cancer patients may be related to the inflammatory response even though it remains debated (Helsby et al., 2008; Burns et al., 2014; Yasu et al., 2017; Mafuru et al., 2019). Other authors showed that cancer has no impact on CYP1A2 metabolic activity as compared to liver disease or infection (Wang et al., 2010).

Therapies With Immunomodulator, anti-TNF-α and -Mabs

As biological therapies aim to decrease the underlying inflammation of the disease, interleukins (IL) injections are expected to have an impact on CYP activity, as underlined in **Table 13**. As an example, IL-2 doses of $9–12 \times 10^6$ units daily may downregulate CYP activities in patients with HIV infection and cancer in whom this treatment is administered to boost the immune system (Piscitelli et al., 1998; Elkahwaji et al., 1999). Conflicting results exist regarding IFN administration, with a discrepancy between acute and chronic treatment (Williams and Farrell, 1986; Williams et al., 1987; Jonkman et al., 1989; Israel et al., 1993; Hellman et al., 2003; Sulkowski et al., 2005; Gupta et al., 2007; Furlanut et al., 2010; Brennan et al., 2013). However, case reports and more specific studies assessing CYP metabolic activity lean toward CYP downregulation and care must be taken to avoid interactions and ADRs (Craig et al., 1993; Adachi et al., 1995; Serratrice et al., 1998; Hassan et al., 1999; Becquemont et al., 2002). The level of anticoagulation should be closely monitored when interferon is given together with warfarin, as it appears that CYP are downregulated (Adachi et al., 1995; Serratrice et al., 1998). Additionally, the timing of IFN-α administration relative to concomitant chemotherapy should be considered to avoid a decrease in CYP3A4 and 2B6 activity and thus to achieve better efficacy (Hassan et al., 1999). For example, interferon-α-2b inhibits CYP1A2, 2D6 and 2C19 and these findings pose new challenges for patients on these therapies with respect to PK interaction with concomitant drugs commonly used (Islam et al., 2002). Further studies are needed to measure the impact of IFN and new cytokine therapies coming on the market on CYP activities. Cytokines act on CYP in an isoform-specific manner, and it is likely that IFN or IL modulate different CYP while they have no impact on others. Moreover, it is crucial to understand whether the modulation of CYP activity is due to this kind of therapy, to the underlying disease which may be inflammatory,

TABLE 13 | Impact of therapies with immunomodulator on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Treatment with IL-2	indinavir (CYP3A)	8 = HIV seropositive patients (observational), 9 = HIV seropositive patients (prospective)	- in the HIV seropositive-patients, the mean concentration of indinavir was significantly increased on day 5 of IL-2 therapy, - in the nine HIV seropositive-patients, the mean indinavir AUC increased significantly by 88% between day 1 and day 5 of IL-2, - mean IL-6 concentrations during IL-2 therapy increased between day1 and day5 from 4- to 86-fold, - study combines observations made in one observational and one prospective (as part of a phase II trial) studies	Williams and Farrell (1986), Cohort study and non-randomized
Treatment with IL-2	/	5 = 3 or $6x10^6/m^2$ units of IL-2, 6 = 9 or $12x10^6/m^2$ units of IL-2, 7 = 0 units of IL-2, Patients with cancer	- in non-tumorous liver fragment removed with the tumor in each patients, authors observed that CYPs proteins (CYP1A2, 2C, 2E1 and 3A), monooxygenase activities of methoxyresorufin and erythromycin and total CYPs were significantly decreased only in the group of patients treated with highest doses of IL-2, compared to control	Furlanut et al. (2010), Randomized clinical trial
Treatment with IFN-α	theophylline (CYP1A2), antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A), hexobarbitone (CYP2C19)	7	- no significant difference in TNF-α, IL-1β, IL-6 and CRP activities after both acute (initiation) and chronic (2 weeks) IFN-α injections compared to baseline, except for TNF-α activity that significantly decreased after chronic therapy, - significant effects of acute IFN-α administration on the oral clearance of the three probe drugs were not detected, - chronic exposure to IFN-α was associated with a significant lowering clearance (33% compared with baseline, $p < 0.05$) but no significant correlations were observed between the changes in theophylline clearance and changes in serum cytokines or acute phase proteins, - chronic IFN-α therapy decreased antipyrine oral clearances by 20% but this did not reach statistical significance and it appeared to have no effect on the metabolism of racemic hexobarbitone	Sulkowski et al. (2005), Cohort study
Treatment with IFN-α	aminophylline (CYP1A2)	12 = healthy volunteers	- after IFN-α treatment in healthy volunteers, there were significant 10–15% increases ($p < 0.05$) in the terminal elimination half-life and AUC of aminophylline administered intravenously, - the total clearance showed a comparable decrease ($p < 0.05$)	Gupta et al. (2007), Non-randomized

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TABLE 13 | (*Continued*) Impact of therapies with immunomodulator on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Treatment with IFN	theophylline (CYP1A2)	5 = hepatitis B, 4 = healthy subjects	- a reduction of theophylline elimination was observed in 8 subjects (remaining subject was a healthy control) and was ranged from 33 to 81%, compared to initial theophylline clearance study, - no impact of the hepatitis on these results because there was no clinical or biochemical change in the liver disease, - a second theophylline clearance study was done 4 weeks after the interferon's injection and it was back to initial value	Hellman et al. (2003), Non-randomized
Treatment with IFN-α	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A)	5 = hepatitis B, 4 = healthy subjects	recombinant leukocyte α-interferon reduced the antipyrine clearance by 16% ($p < 0.01$) and the half-life increased but this was not significant	Brennan et al. (2013), Non-randomized
Treatment with IFN-α	warfarin (CYP2C9)	52 year-old-woman	- her prothrombin time increased to 16.7–20.4 s with a rise in serum warfarin concentration from <0.8 μg/ml to 5.2 μg/ml 10 days after the onset of IFN-α therapy, - dose was reduced and both anticoagulation and serum warfarin concentration had returned to nearly baseline values	Adachi et al. (1995), Case report
Treatment with IFN-α-2b	acenocoumarol (CYP2C9)	46-year-old-woman	- at the beginning of the treatment, anticoagulant effect of acenocoumarol increased (thrombotest decreased from 30–35–19%), - when IFN-α-2b dosage decreased because of infection remission, anticoagulant effect decreased (thrombotest increased from 25–40–69%), - it led to the adaptation of the dosage of acenocoumarol to be on thrombotest range, - anticoagulation level decreased from 1 day after injection to 2 or 3 days later	Serratrice et al. (1998), Case report
Treatment with IFN-α-2b	ERMBT (CYP3A)	6 = chronic hepatitis C, 4 = healthy controls	- ERMBT before and 20–26 h after IFN-α-2b injection, - IFN-α-2b induced a small significant decrease in ERMBT ($p < 0.05$), - at baseline CYP3A4 activity was lower in patients with hepatitis C but the effect of IFN appeared to be not different	Craig et al. (1993), Non-randomized
Treatment with IFN-α	cyclophosphamide (CP) (CYP2B6 active metabolite and CYP2C9, 2C19 and 3A substrate)	10	- administration of IFN-α before CP caused a 63% decrease in its clearance ($p = 0.004$) compared to an administration of IFN-α 24 h after CP, - there is a 45% decrease in exposure of CP active metabolite's (4-OHCP) when IFN-α was administered before CP, expressed as AUC ($p = 0.002$), compared with that observed when IFN-α was administered 24H after CP, - this resulting in a greater decrease in leukocyte count (45%, $p = 0.02$) when IFN-α was given after CP in the 10 patients with multiple myeloma	Hassan et al. (1999), RCT

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TABLE 13 | (*Continued*) Impact of therapies with immunomodulator on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Treatment with IFN-α-ribavirin	dextromethorphan (CYP3A4 and CYP2D6, by measuring different metabolite) and caffeine (CYP1A2)	14	- mean CYP3A4 activity increased from 0.18 ± 0.06 in patient with HCV before beginning of IFN-α-ribavirin treatment to 0.48 ± 0.53 1 month after but this did not reach statistical significance (p = 0.19) - a similar evolution of CYP2D6 activity could be observed during the first month of treatment (148 ± 0139 to 421 ± 641, p = 0.08), - CYP1A2 activity did not changed, going from 0.39 ± 0.11 before treatment to 0.32 ± 0.13 after 1 month, - pretreatment CYP3A4 and CYP2D6 activities of the 14 studied patients were significantly lower than those observed in 35 healthy volunteers (p = 0.0006 and p = 0.0008 respectively), - after 1 month of antiviral treatment, CYP3A4 and 2D6 did not differ significantly from those in healthy volunteers, probably because of the recovery of HCV patients	Becquemont et al. (2002), Non-randomized
Treatment with IFN-α-2b	caffeine (CYP1A2), mephenytoin (CYP2C19), debrisoquin (CYP2D6), chlorzoxazone (CYP2E1) and dapsone (CYP2C8 and CYP2C9)	17 = patients with high-risk resected melanoma	- IFN-α-2b inhibits immediately the activity of CYP1A2 (p = 0.001) and 2D6 (p < 0.001) in patients with high-risk resected melanoma, - inhibition of CYP2C19 was detected for the first time at day 26 (p < 0.001) after the initiation of high-dose IFNα-2b treatment (20 MU/m2/day i.v for 5 days/weeks during 4 weeks and 10 U/m2/day s.c for 3 days/week x 48 weeks), - no significant inhibition was seen for CYP2E1	Islam et al. (2002), Cohort study
Treatment with peginterferon-α-2b	dextromethorphan (CYP2D6) and, fluoxetine (CYP2D6 active metabolite)	20	- MR before and after initiation of peginterferon-α-2b and ribavirin therapy go from 0.10 ± 0.40 to 0.04 ± 0.09 and that's mean that metabolite production of dextromethorphan increased after hepatitis C, but it is not significant (p = 0.087), - mean serum concentrations of fluoxetine and its metabolite (norfluoxetine) at baseline and 2 months later during combined antiviral treatment didn't change significantly, - only the half-life of fluoxetine showed a significant reduction during combined antiviral therapy (p = 0.014)	National Center for Biotechnology Information (2012), Cohort study
Treatment with peginterferon-α-2a	methadone (CYP3A, 2C8 and 2D6)	24 with hepatitis C	- treatment did not alter the pharmacokinetic of methadone in patients, - increase exposure of total methadone by 10–15% was not statistically significant	Wu and Fleming (2011), Non-randomized
Treatment with peginterferon-α-2b	methadone (CYP3A, 2C8 and 2D6)	20 with hepatitis C	- a barely significant increase in total methadone exposure of 15–16% was observed after 4 weekly injection of peginterferon-α-2b - this increase was not clinically significant because there were no symptoms of methadone overdose	Ling et al. (2009), Non-randomized

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TABLE 13 | (*Continued*) Impact of therapies with immunomodulator on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Treatment with peginterferon-α-2a	theophylline (CYP1A2), tolbutamide (CYP2C9), mephenytoin (CYP2C19), debrisoquin (CYP2D6) and dapsone (CYP3A)	14	- theophylline AUC increased significantly but Cl/F difference was not significant, - no effect on the PK of any other probe drug	Schmitt et al. (2011), Cohort study
Treatment with INF-β	mephenytoin (CYP2C9 and 2C19 and induces 2C9, 2C19 and 3 A) and debrisoquin (CYP2D6)	10 with multiple sclerosis in the first stage	(S)/(R) mephenytoin ratio (p = 0.5) and debrisoquine MR (p = 0.4) were not statistically significant different before and during regular INF-β treatment	Zhuang et al. (2015), Non-randomized

or to its resolution by these same therapies (reduction of inflammation caused by the disease).

The impact of–mabs therapies are summarized in **Table 14**. Monoclonal antibodies have a high degree of specificity against an antigen or an epitope (National Center for Biotechnology Information, 2012). In 2018, more than sixty therapeutic monoclonal antibodies were approved and used in the United States for their action against specific immune cells such as lymphocytes and cytokines or against specific enzymes, cell surface transporters or signaling molecules (National Center for Biotechnology Information, 2012). Consequently, a number of studies have examined the impact of monoclonal antibodies on CYP metabolic activity, assuming that these drugs, by reducing inflammation, return CYP metabolic activity to baseline (Ling et al., 2009; Schmitt et al., 2011; Wu and Fleming, 2011; Zhuang et al., 2015; Tran et al., 2016; Lee et al., 2017; Wen et al., 2020) (**Table 14**).

A return to baseline level after treatment of inflammation was not always observed (Wollmann et al., 2017; Davis et al., 2018). A lag was observed in some cases, such as basiliximab coadministration, which increased tacrolimus through concentration on day 3 but decreased on day 30 (Sifontis et al., 2002). Moreover, OKT3 (also known as muromonab, a CD3 receptor antibody) treatment transiently increased CyA through concentration, and authors suggested that OKT3 inhibits CYP3A4 metabolic activity by inducing transient cytokine release (Vasquez and Pollak, 1997). No changes were observed in drugs PK parameters before and after monoclonal antibodies administration, possibly because CYP metabolic activity was similar in psoriasis disease and in healthy volunteers (Bruin et al., 2019; Khatri et al., 2019). However, these therapies are used for a variety of diseases, with different levels of proinflammatory markers. In addition, a recently published study assessed the impact of clazakizumab, an anti-IL-6 antibody, in kidney transplant recipients with antibody-mediated rejection (ABMR) on CYP3A and CYP2C19 activity by pantoprazole and on tacrolimus and CyA concentrations (Mühlbacher et al., 2021). In contrast to earlier observations, prolonged blockade of IL-6 did not enhance CYP metabolism (Mühlbacher et al., 2021). This could be because the included patients did not have systemic inflammation before initiation of clazakizumab, with IL-6 and CRP levels in the normal range (Mühlbacher et al., 2021). Thus, clazakizumab did not increase CYP metabolism because the included patients had unaltered CYP expression, as ABMR may be different from other disease states, such as infection or autoimmune disease, where systemic inflammation is present (Mühlbacher et al., 2021).

DISCUSSION AND PERSPECTIVES

Our systematic review identified 218 publications that evaluated the impact of inflammation on CYP activities which we divided into 17 sources of inflammation. Indeed, current literature suggests that cytokine signalling pathways differ according to the trigger of inflammation, leading to heterogeneous effects on CYP activity, with different magnitude, potency and time-course (de Jong et al., 2020; Stanke-Labesque et al., 2020). This analysis allowed us to identify areas where the literature is abundant, such as infections like pulmonary infection, hepatitis or HIV and for some therapeutic agents like immunosuppressants or clozapine, and others where further research is needed, such as for auto-immune diseases, and other specific diseases such as diabetes or the anti-inflammation treatments.

Our analysis also identified that studies should be more specifically conducted to assess whether resolution of inflammatory episodes allows a return to baseline of CYP activities. Indeed, inflammatory diseases are chronic, but with a possibility of remission, and acute inflammatory events can punctuate life (infection, surgery, cancer…). A better understanding of the mechanisms of modulation and return to the initial state would make it possible to anticipate changes in the PK of concomitant treatments at different phases of the disease or of the patient's life. This could be done through the impact of anti-inflammatory treatments as well as monoclonal antibody therapies. These therapies are relatively new and much remains to be discovered, but they are highly targeted, and the impact of these different molecules could be isoform specific.

Our literature review highlighted the different effect of inflammation according to the CYP considered. Several studies have investigated the impact of infection on drugs of the nervous systems, mainly CYP2D6 substrates without always showing a significant impact. It now appears that CYP2D6 activity is not modulated by inflammation and this is confirmed in chronic hepatitis C patients where downregulation is linked to the presence of liver kidney microsomal type 1 (LKM-1) antibodies (Girardin et al., 2012). LKM-1 antibodies are often produced during chronic HCV infection and appear to be

TABLE 14 | Impact of therapies with anti-TNF-α and -mabs on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Basiliximab	tacrolimus (CYP3A)	12 = treatment, 8 = control	- 63% increased tacrolimus trough concentration in basiliximab group at day 3 vs controls ($p < 0.05$), - tacrolimus through concentration decreased in basiliximab group 30 days after transplantation, - Authors suggest that basiliximab induced alteration in drug metabolism because its binding to IL-2R on activated T cells allows circulating IL-2 to bind to IL-2R on hepatic and intestinal cells resulting in a down-regulation of CYP3A4	Wen et al. (2020), Non-randomized
OKT3 (muromonab)	CyA (CYP3A)	17 = OKT3, 16 = controls	- on days 1 and 3, CyA through concentration did not differ but it was significantly higher in OKT3-group at day 5 as compared to control ($p < 0.0001$), - on days 7 and 10, CyA through level did not differ again	Tran et al. (2016), Case-control study
Adalimumab	duloxetine (CYP1A2 and 2D6)	22 years-old woman	- adalimumab was initiated for a refractory psoriasis but the peripheral neuropathy became unbearable leading to double the duloxetine's dosage while she had a long-standing treatment by duloxetine and pregabalin, - authors did not suggest any interaction's mechanism but it could be possible that the decrease of TNF-α by adalimumab led to a lift of the inhibition of CYPs, - no apparent interaction with pregabalin, which is eliminate by renal way	Lee et al. (2017), Case report
Infliximab	verapamil (CYP3A4, 1A2, 2C8, 2C9 and 2C18)	12 = RA with infliximab, 8 = RA controls, 12 = healthy controls	- serum CRP and IL-6 concentrations were significantly greater in RA patients who were on nonbiologic antirheumatic therapy compared with controls ($p < 0.05$ and $p < 0.001$, respectively), - CRP and IL-6 concentrations were not significantly different between RA patients taking infliximab and control subjects, - difference in RA patients who were on nonbiologic treatment in all PK parameters of verapamil, but it did not reach statistical significance but no difference between controls and RA patients who were taking infliximab, - infliximab did not show overall superiority to placebo on depressive symptom outcome	Davis et al. (2018), Case-control study
Infliximab	antidepressants	30 = infliximab, 30 = placebo		Wollmann et al. (2017), RCT
Secukinumab	midazolam (CYP3A)	24 = Psoriasis Area Severity Index (PASI) score >12 taking secukinumab	- secukinumab treat the immune-mediated disease by neutralizing the underlying inflammation and tissue destruction, - patients with PASI score >12 taking secukinumab, a decreased in IL-6 and CRP levels were observed after the start of treatment, - any change was seen in the PK parameters of midazolam before and after the administration of secukinumab, - PK parameters of midazolam in patients with psoriasis (study subjects) were close to those in found in healthy subjects in a previous study	Sifontis et al. (2002), Non-randomized

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TABLE 14 | (*Continued*) Impact of therapies with anti-TNF-α and -mabs on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
risankizumab	caffeine (CYP1A2), warfarin (CYP2C9), omeprazole (CYP2C19) and metoprolol (CYP2D6)	21	- risankizumab is an antibody that acts against IL-23 and it is involved in immune and inflammatory response thus, risankizumab inhibits its cells signalling pathway and the release of pro-inflammatory cytokines, - metabolic activity of CYP1A2, 2C9, 2C19, 2D6 and 3A4 were assessed before and 12 weeks after onset of treatment and any differences were observed, - authors conclude that treatment with risankizumab is not expected to cause CYP-mediated drug interactions	Vasquez and Pollak (1997), Non-randomized
tocilizumab	simvastatin (CYP3A)	12	- exposure to simvastatin was significantly reduced by approximately half at 1 and 5 weeks after tocilizumab infusion	Bruin et al. (2019), Randomized
sirukumab	midazolam (CYP3A), omeprazole (CYP2C19), warfarin (CYP2C9), caffeine (CYP1A2)	12	- administration of probe drugs 1 week before and 1, 3 and 6 weeks after sirukumab administration, - AUC of midazolam, omeprazole and S-warfarin decreased and those of caffeine increased as compared with those before sirukumab administration, - it was not because it is a CYP inducers, but because the inhibition by inflammation may be reversed by its IL-6 antagonism, - for CYP1A2, this result suggests that inflammation induce its metabolic activity, - authors suggest that, according to literature, IL-6 may have a biphasic impact on CYP1A2 activity depending on the IL-6 concentration, with an induction observed with low level of IL-6	Khatri et al. (2019), Non-randomized
dupilimumab	midazolam (CYP3A), omeprazole (CYP2C19), warfarin (CYP2C9), caffeine (CYP1A2) and metoprolol (CYP2D6)	13	- no impact of blockade of IL-4 and IL-13 signalling on the metabolic activity of CYP3A, 2C19, 2C9, 1A2 and 2D6	Mühlbacher et al. (2021), Non-randomized
biological disease-modifying antirheumatic drugs	4β-hydroxycholesterol (4βOHC) (CYP3A)	31 = TNF-α inhibitor, 5 = IL-6 inhibitor, 5 = B-cells inhibitors, 52 = controls	- levels did not change after the onset of any of the three treatments, - a trend was observed that lowest baseline 4βOHC levels (higher inhibition of CYP3A4 metabolic activity) showed highest relative increase in at follow-up and thus a highest regain in metabolic activity of CYP3A4 after initiation of treatment, - authors suggest that the absence of variation in 4βOHC levels in this study could be explained by the low level of inflammation in these patients because 4βOHC level in the study population at baseline was only 30% lower than in control groups	Girardin et al. (2012), Cohort study and case-control study
TNF-α inhibitor	4βOHC (CYP3A)	31	- CRP values were lower than before 3 months treatment, but the difference was not statistically significant ($p > 0.2$) and 4βOHC levels were not significantly affected ($p > 0.9$) by the initiation of treatment, - significant negative correlations were observed between 4βOHC and IL-1ra and IL-6 ($r = -0.410$, $p = 0.022$) and CXCL8 ($r = -0.403$, $p = 0.025$)	Chládek et al. (1999), Cohort study Same subject as in Girardin et al. (2012)

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TABLE 14 | (*Continued*) Impact of therapies with anti-TNF-α and -mabs on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
etanercept	CyA (CYP3A)	42-year-old male	-2.5-fold increase of clearance after initiation of etanercept	Yang et al. (2003), Case-report
daclizumab	caffeine (CYP1A2), warfarin (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6) and midazolam (CYP3A)	30 = multiple sclerosis	- daclizumab treatment had no effect on CYP1A2, 3C9, 2C19, 2D6 and 3 A activity in patients with multiple sclerosis as compared to before treatment	Hefner et al. (2015), Cohort study
sarilumab	Simvastatin (CYP3A)	19	- plasma exposure decreased by 45% in RA patients 1 week after sarilumab injection, as compared to baseline, - one dose led to decreased of CRP level and IL-6 inhibition and, thus, restauration of CYP3A enzyme activity	Harbrecht et al. (2005), Cohort study

proportional to liver disease severity (Girardin et al., 2012). Moreover, it is well-known that CYP2D6 has an important inter- and intra-individual variability, in accordance with the available literature (Chládek et al., 1999). All sources of inflammation combined, the most studied CYP was CYP3A, which is in fact the CYP that metabolizes nearly 50% of the drugs on the market. Patients with inflammation/infection are, however, prone to receiving multiple drugs, and the impact on other CYPs should be carefully evaluated, in particular in critically ill patients or patients at different stages of HIV, where data is scarce. Studies should also be careful to exclude the impact of co-medications (CYP inhibitor and inducer) as a confounding factor.

In organ diseases, current studies in liver diseases have not been able to determine whether CYP downregulation is caused by a decrease of CYP content or not, and in renal diseases it was not possible to identify whether the modulation of CYP activity was rather due to elimination issues (Farrell et al., 1979; Yang et al., 2003). Therefore, it is challenging to study inflammation as an independent factor in PK variability and not as a consequences of organ damage.

Our literature review also found that inflammation is a complex process, which is expressed differently depending on the disease and conditions and therefore, extrapolation between different types of inflammation should be avoided. Indeed, the hepatic expression of CYP2C19 could for example be regulated by other tumor-associated inflammatory factors than those regulating CYP3A (Burns et al., 2014). Moreover, different levels of inflammation led to different magnitudes of voriconazole through concentration increases for instance in association with CRP levels (van Wanrooy et al., 2014; Bolcato et al., 2021). In most studies, significant changes in CYP activities occurred in the presence of severe inflammation, characterized by elevated levels of inflammatory markers or a severe disease state, such as AIDS, advanced cancer or polytrauma patients (Gatti et al., 1993; Lee et al., 1993; Farrell et al., 1979; Grieco et al., 1998; Bauer et al., 1994; Harbrecht et al., 2005; Charles et al., 2006; Alexandre et al., 2007; Helsby et al., 2008; Abou Farha et al., 2012; ten Bokum et al., 2015; Hefner et al., 2015; Yasu et al., 2017; Gautier-Veyret et al., 2019). A minority of studies have evaluated the impact of inflammation on drugs PK and metabolism as an independent factor of variability, as only a few have included inflammation factors as covariates, such as biomarkers of renal or liver function (Stanke-Labesque et al., 2020).

Additionally, inflammation may have a different impact on CYPs activities depending on their baseline activity and on genotypic and environmental factors, such has concomitant treatments. Indeed, inflammation further increased the perampanel concentration/dose (C/D) ratio in patients not treated with drug inducers (Yamamoto et al., 2018). Voriconazole is also metabolized by highly polymorphic CYPs and inflammatory marker levels have a differential impact on voriconazole trough concentration whether patients are extensive, intermediate or ultra-rapid metabolized for CYP2C19 (Veringa et al., 2017). Moreover, a recent meta-analysis showed that voriconazole trough concentrations were independently influenced by both CYP2C19 and CYP3A4 genotype, considered individually or by a combined genetic score, in addition to CRP levels (Bolcato et al., 2021). In contrast, another cohort study showed that voriconazole overdoses were significantly associated with elevated CRP levels (>96 mg/L) but that CYP2C19 and CYP3A4 genotype, considered alone or combined in a genetic score, were not significantly different between overdose and non-overdose patients (Gautier-Veyret et al., 2019). Therefore, inflammation and pharmacogenomics may mutually minimize their reciprocal influence on CYP phenotype. Indeed, genotype did not predict correctly the phenotype in patients with inflammatory disease and the effect of inflammation was not as important as expected in CYP variants carriers (Helsby et al., 2008; Goktaş et al., 2015; Burns et al., 2014; O'Neil et al., 2000; Williams et al., 2000;). Consequently, inflammation could induce dynamic phenoconversion, characterized by dynamic phenotype-genotype mismatch, and studies examining the impact of inflammation on CYPs should assess CYP genotypes and phenotypes as covariates. It should however be pointed out that most of the included studies did not take into account routine treatment given to treat the diseases themselves.

Predictive models based on known interactions between molecular, environmental and lifestyle data by computational algorithm are increasingly developed to support the decision to individualize treatment (Iriart, 2019). Simulation of the

concentration-time profiles of a drug and its metabolite(s) and concomitant estimation of PK parameters using dynamic physiologically based pharmacokinetic (PBPK) models allow prediction of plasma concentration curves (Sager et al., 2015). There are increasing developments in regulatory guidances (Sager et al., 2015). Inflammatory disease is an example of a special population and numerous PBPK models have been developed and validated to predict IL-6 mediated drug-disease (Machavaram et al., 2013; Xu et al., 2015; Jiang et al., 2016; Radke et al., 2017; Xu et al., 2018; Machavaram et al., 2019). While IL-6 appears to be the key element in modulating CYP activities during inflammation, a recent study developed a model that predicted the impact of systemic CRP levels on CYP3A4 and CYP2C19 activities (Simon et al., 2021). Optimal drug use leads to takes into account the contribution of covariates to predict the dose needed to achieve a target concentration and thus reduce the inter- and intra-individual variability in drug response (Darwich et al., 2021).

This review focuses on CYP regulation, but other mechanisms, such as enzymes and transporters, involved in drug absorption, distribution, metabolism and elimination may be involved in changes in drugs PK during inflammatory states, although they are less studied. Studies described changes in plasma protein binding and renal excretion during inflammation that could affect CYP substrates metabolism (Gorski et al., 2000; Hefner et al., 2015; Helland et al., 2018). Plasma protein binding may influence total clearance for low-extraction drugs but not unbound clearance and may or may not influence half-life, depending on clearance and volume of distribution (Boffito et al., 2021). The unbound concentration and not the total concentration must be considered when assessing drug exposure to a highly protein-bound drug, otherwise there is a risk of misinterpretation of lopinavir overexposure (Boffito et al., 2021; Stanke-Labesque et al., 2021). For example, by taking into account plasma protein concentration, the authors concluded that CyA biotransformation by CYP3A may be downregulated by diabetes (Akhlaghi et al., 2012). Decreased albumin concentration may increase the unbound concentration in diabetics, which should theoretically increase CyA metabolic clearance (Akhlaghi et al., 2012). But the lower production of almost all metabolites has shown that the correct hypothesis is rather a reduced CYP activity (Akhlaghi et al., 2012). In fact, CyA metabolites that involved amino acid 1 showed significantly lower dose-normalized AUC values in diabetic patients compared with nondiabetics suggesting that CYP3A4 metabolic activity was not decreased (Mendonza et al., 2008). Its dose-adjusted metabolite-parent concentration ratio was decreased in the diabetic groups, but no difference was found concerning doses and trough levels of CyA in a retrospective study (Wadhawan et al., 2000; Akhlaghi et al., 2012).

Phase 2 drug metabolic enzymes appear to be affected in a cytokine-specific manner, as infection resulted in a significant downregulation of several genes encoding hepatic uridine 5′-diphospho-glucuronosyltransferases (UGT) (Stanke-Labesque et al., 2020). Pregnane X receptor (PXR) and constitutive androstane receptor (CAR), two nuclear receptors, are also cytokine dependent and mediate the expression of glutathione S-transferases (GST), UGTs and sulfo-transferases (SULT) in humans (Wu and Lin, 2019). However, unlike voriconazole, posaconazole's PK did not appear to be influenced by inflammation. This could be explained by a metabolism by phase 2 enzymes mainly (Märtson et al., 2019). Literature reviews on physiological changes related to drug PK and PD during inflammation may be useful to determine what investigations are needed to complement the data in the literature, such as the impact of inflammation on P-gp and other drug transporters, as one study showed that an increase in bioavailability due to downregulation of P-gp could not be ruled out (Sanaee et al., 2011).

Moreover, hepatic transporters that belong to ATP-binding cassette (ABC) and solute carrier (SLC) transporters have been shown to be significantly reduced during inflammatory states in animal and in-vitro studies (Stanke-Labesque et al., 2020). For instance, animals studies have shown that mRNA levels of MRP, OATP or BSEP were decreased in mice during inflammation (Wu and Lin, 2019). NF-κB, a transcription factors involved in the mechanism of action of cytokines on metabolizing enzyme gene expression, is also known to regulate the expression of numerous ABC and SLC transporters, including ABCB1 in humans and MDR1, MRP, BCRP, OATP, NTCP in rats and mice (Wu and Lin, 2019).

Given all of the above, it should be acknowledged that our literature search has some limitations. First, the completeness of the search cannot be guaranteed as we only searched one database and only published articles. Second, there is inevitably heterogeneity between the studies selected due to the different methodologies employed and low comparability between the studies identified. In addition, the diversity of the sources of inflammation studied and assessment of the clinical impact severity limits the robustness and generalizability of the results. Interpretations should therefore be addressed with particular caution.

CONCLUSION

This systematic literature review shows that inflammation is a major contributing factor to CYP metabolic activity variations. The proportion of the drug cleared by CYP metabolism, the patient's genotype and concomitant medications should also be taken into account.

Compelling evidence suggests that inflammation has a differential impact on the various CYP isoforms with a different magnitude. CYP3A and CYP2C19 are downregulated and inflammation has no impact on CYP2D6 activity. Regarding other main CYPs, the impact remains unclear and requires further investigation. Moreover, the effect of inflammation depends on its severity and the inflammatory markers released, even if this remains debated. Indeed, the origin of the inflammation may differ as well as the inflammatory mediators involved, possibly leading to different impact on CYP activities. The reason why some CYP metabolic activities were modulated in some diseases and not in others may be partly explained by this heterogeneity in inflammatory markers.

Nonetheless, some results are still debated such as the impact of vaccination and infection, and further investigations are required to well characterize the impact of inflammation on CYP activity.

CYP is a major source of interindividual variability, and it appears crucial to be able to predict their activity to individualize drug dosing and take into account the patient's underlying pathophysiological conditions and the PK characteristics of the drug concerned. Measurement of inflammation induced CYP phenoconversion and the development of endogenous markers of CYP metabolism should enable the measurement of CYP activity variation due to disease progression and could have implications for personalized medicine and provide new opportunities.

To conclude, inflammatory conditions in patients are a major factor to be considered to predict variability in drug response and avoid efficacy or safety issue in clinical practice.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

CL participated in the manuscript conceptualization, experimental design, writing and data analysis. CFS, JAD and VR participated in the manuscript conceptualization, supervision, overall manuscript review and English review.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Chapter 7: Impact of Inflammation on Cytochromes P450 Activity in Pediatrics: A Systematic Review.

Summary

Chapter 6 evidenced that inflammation contributes to the intra- and inter-individual variabilities in CYPs expression and activity. For reminder, **chapter 1** explained that children are also a special population due to ontogeny. Consequently, pediatric populations cannot be described by a single uniform pattern and data used cannot exclusively come from the extrapolation of adult studies. Therefore, inflammation will probably not have the same impact according to age.

Chapter 7 aims to evaluate the impact of inflammation on CYPs activity in the pediatric population to support precision medicine. The **review article 4**, published in *Clinical Pharmacokinetics*, is a systematic review that summarized and classified by CYPs isoforms the drug-disease interactions found in 27 studies and case reports. Similarly to adults, data suggest that the impact of inflammation is isoform-specific, depending on the intensity and the nature of the disease. In contrast to adults, the amount of CYPs isoforms depends on the developmental stage, which leads to a different impact of inflammation on CYPs activity and expression depending on age. Furthermore, it should be noted that almost no studies have been conducted in the periods of greatest developmental changes (neonatal and early infancy) and on CYPs other than CYP3A and CYP1A2.

My contributions to this **review article 4** were the participation in the manuscript conceptualization, experimental design, systematic research, data analysis, and writing the article.

Review article 4: Impact of Inflammation on Cytochromes P450 Activity in Pediatrics: A Systematic Review.

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SYSTEMATIC REVIEW

Impact of Inflammation on Cytochromes P450 Activity in Pediatrics: A Systematic Review

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Abstract

Background and Objective Cytochromes P450 (CYP) are the major enzymes involved in hepatic metabolism of drugs. Personalization of treatment in pediatrics is a major challenge, as it must not only take into account genetic, environmental, and physiological factors but also ontogeny. Published data in adults show that inflammation had an isoform-specific impact on CYP activities and we aimed to evaluate this impact in the pediatric population.

Methods Articles listed in PubMed through 7 January, 2021 that studied the impact of inflammation on CYP activities in pediatrics were included in this systematic review. Sources of inflammation, victim drugs (CYP involved), effect of drug–disease interactions, number and age of subjects, and study design were extracted.

Results Twenty-seven studies and case reports were included. The impact of inflammation on CYP activities appeared to be age dependent and isoform-specific, with some drug–disease interactions having significant pharmacokinetic and clinical impact. For example, midazolam clearance decreases by 70%, while immunosuppressant and theophylline concentrations increase three-fold and two-fold with intensive care unit admission and infection. Cytochrome P450 activity appears to return to baseline level when the disease is resolved.

Conclusions Studies that have assessed the impact of inflammation on CYP activity are lacking in pediatrics, yet it is a major factor to consider to improve drug efficacy or safety. The scarce current data show that the impact of inflammation is isoform and age dependent. An effort must be made to improve the understanding of the impact of inflammation on CYP activities in children to better individualize treatment.

1 Introduction

Inflammation is a universal protective reaction to endogenous or exogenous aggression that involves all tissues and both innate and adaptive immunity. It is known to induce changes in the concentrations of many plasma proteins and in several behavioral, physiological, and biochemical mechanisms [1]. Inflammation is complex and well orchestrated, as certain triggered mechanisms initiate, amplify, or sustain the process with many cell types and molecules [1]. Cytokines, and in particular interleukin-6, are the main stimulators of these acute changes [1]. Published data in adults indicate that inflammation has an impact on cytochromes P450 (CYP) activity, the major enzymes involved in drug metabolism, in an isoform-specific manner, and as a result of pre-transcriptional and post-transcriptional mechanisms that are cytokine specific [2–7]. Indeed, CYP activity is influenced by the interaction of genetic, environmental, and physiological factors through a wide variety of ligand-activated transcription factors and mediators regulating hepatic CYP content [6, 8]. Understanding the impact of inflammation on CYP activity is important to understand in order to personalize drug use, as many diseases such as infection, cancer, diabetes mellitus, autoimmune disease, surgery, or trauma are associated with inflammation [1, 9, 10].

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Key Points

The impact of inflammation on cytochrome P450 activities appears to be age dependent in the study population.

The impact of inflammation on cytochrome P450 activities appears to be isoform-specific.

Data that have evaluated the impact of inflammation on cytochrome P450 activities in pediatrics are lacking, as they frequently are in this particular population.

Children are not exempt from inflammation and inflammatory diseases, but data are scarce on the impact of inflammation on CYP activities and drug metabolism in the pediatric population [11]. It is well known that pediatric clinical trials are often lacking and less than half of labelled drugs have pediatric data [12]. Moral, ethical, and legal issues prevent rigorous scientific investigations in the pediatric population, and infant dosing regimens are often extrapolated based on data available only in the adult population [13]. However, children differ from adults in terms of height and weight but also in physiological perspectives because of an ontogeny [12]. The maturation and development of organs and enzyme systems influence the pharmacokinetics (PK) and pharmacodynamics of drugs, which may lead to potential variation in the efficacy and safety of drugs [13]. Ontogeny processes are complex and non-linear, making the pediatric population very heterogeneous and as such, the developmental course of all processes contributing to drug disposition cannot be described by a single uniform pattern [14, 15]. However, differences in drug-metabolizing enzyme activity appear to be the main determinants of the overall pharmacokinetic differences observed between adults and children [16]. Cytochrome P450s are mostly present at birth but are immature [15]. The development of enzyme activity over time is isoform-specific and is rapidly improving in the first weeks/years of life [12, 15]. Although data are still sporadic and sometimes contradictory, it is generally recognized that CYP1A2 has the slowest developmental pattern [17–19]. CYP2C19 and CYP3A4 likely have an intermediate pattern, with an adult's activity reached at the end of infancy [17–19]. In contrast, CYP2B6, CYP2C9, and CYP2D6 activity increases rapidly during the first months of life and early infancy [17–19]. As with adults, the use of effective and safe therapy in children requires a good understanding of the inter-individual and intra-individual variability due to their growth and maturation, and ontogeny should be taken into account when selecting a drug dosage in children [15, 17, 20]. Many efforts have been made in recent decades to predict age-related alterations in the PK of drugs in children [14]. Modeling approaches, such as physiology-based PK, are increasingly used in order to obtain pediatric data including both growth and maturation processes (intrinsic characteristics) and drug-specific parameters (extrinsic parameters) [16, 21]. They allow for safe and effective pediatric study designs and successful prediction of PK in the pediatric population [21]. Knowledge of the impact of disease and inflammation on CYP activity and drug PK appears to be an additional important element to consider. The aim of this systemic review was thus to evaluate the impact of inflammation on CYP activity in the pediatric population.

2 Methods

The Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement requirements and the PICOS framework were used to manage and to develop the current literature search, respectively [22]. The PICOS framework we used was as follows: participants: children with source of inflammation; intervention: victim drugs and CYP concerned; comparison: healthy children or before the onset of inflammation or receiving treatment for inflammation; outcomes: effect of the interaction between inflammation and CYP activity; design of the studies: clinical trials and case reports/series.

2.1 Database and Search Strategy

PubMed via MEDLINE, the database of biomedical publications, was used to carry out the literature search for studies and case reports/series until 7 January, 2021. We also completed our literature search with a manual search of references for potentially relevant articles. We used the keywords "Inflammation", "cytochrome P450", "cytochromes P450", and "CYP450".

2.2 Study Selection

The following eligibility criteria were applied to select only pertinent publications from the literature search. Randomized controlled trials, non-randomized studies, and observational studies were the types of studies selected in our literature search, as well as case reports and series. Studies had to be published in English as full-text articles or congress abstracts, from database inception until 7 January, 2021. Studies participants had to be under 18 years of age, including healthy subjects and patients who suffered from inflammatory conditions caused by a disease, treatment, or a medical or surgical procedure. The outcomes of interest were the effect of potential (suggested or provided) inflammation on the metabolic ratios of CYP isoforms and the pharmacokinetic/pharmacodynamic and safety profiles of CYP substrates.

The screening of publications was done in several steps. First, the titles of the articles were read to make an initial selection. Then, the abstract and full text were read successively to filter out potentially relevant articles according to the predefined eligibility criteria. The remaining articles were categorized into literature reviews, in vitro, animals, in silico, and human studies. Studies concerning adults were then removed, retaining only those publications that concerned pediatrics (defined as under 18 years of age). Finally, they were classified as studies or case reports/series. A similar process was applied to the additional articles found by a manual search. The study selection method was summarized in a flowchart created according to the PRISMA statement requirements (Fig. 1) [22].

2.3 Data Extraction and Management

The reference management software Zotero (Version 5.0.85, © 2006-2018 Contributors) was used to collect and export highlighted articles and then, to remove duplicates. Data from the included articles were extracted and synthetized, and the extracted data were classified according to age group, namely pediatrics (under 18 years) and adults (over 18 years). The authors extracted the data according to the PICOS framework previously discussed. As a reminder, these included study design, sample size, source of inflammation and comparators, victim drugs and CYP involved in their mechanism, and outcomes of interests (effect of drug–disease interactions). A check of the metabolite pathway of the victim drug was performed to confirm whether it was a CYP substrate and which CYP was involved. The Summary of Product Characteristics, the Lexi-Interact drug interaction checker, and the Geneva table of CYP substrates, inhibitors, and inducers were used to perform this verification process [23, 24].

3 Results

3.1 Identification and Selection of the Studies

The first step of the PubMed research led to a total of 2283 articles, and of these articles, 523 remained after screening by title and abstract. By cross-referencing and handsearching the reference list of relevant articles, 366 additional articles were added, resulting in 889 articles. Next, 128 records were not available in full text and 224 were not translated into English or considered irrelevant, leading to the deletion of 352 records. The remaining 537 articles were categorized into review articles ($n = 55$), in vitro ($n = 77$), or in-silico ($n = 8$) studies and studies conducted in animals ($n = 152$) or humans ($n = 245$). Only publications involving humans were included in the current systematic review and were classified as including adults ($n = 218$) or children ($n = 27$). Articles and case reports concerning the adult population are the subject of another systematic review. Finally, 27 articles conducted in pediatrics were included and classified as studies ($n = 19$) and case reports/series ($n = 8$) for analysis. These results are summarized in Fig. 1.

3.2 Synthetized Findings

Table 1 summarizes the cases of drug–disease interactions presented in the 27 eligible publications. The drug–disease interactions found in the selected publications were organized by victim drug and CYP involved in their metabolism. The most cited inflammation perpetrator was infection and the two most studied CYPs were CYP1A2 and CYP3A because many were receiving theophylline or immunosuppressants.

4 Discussion

Understanding the PK and the pharmacodynamics of drugs is the key element to accurately determining the safest and most effective dose of a prescribed drug in pediatrics [17]. In children, in addition to environmental, genetic, and individual factors, such as comorbidities and medications, the influence of ontogeny must be considered and complicates prediction of the response to a treatment [17, 20]. However, because of the lack of specific studies, pediatric data are almost exclusively extrapolated from adult studies [15].

One of the covariates known to contribute to dynamic changes in CYP activity in adults is inflammation [2–6]. Little is known about the effect of inflammation on CYP activity in pediatrics and, to our knowledge, there is only one review on the subject [11]. Very few studies have been published in almost 10 years. The consequences of inflammation on CYP activities appear to be different between adults and children and confirms the impossibility of simply extrapolating the adult data, as shown in the different studies included in this review.

4.1 CYP3A4

CYP3A4 was the most studied CYP in children, in particular, the impact of inflammation on tacrolimus and cyclosporin A (CyA) pharmacokinetic parameters was assessed. Tacrolimus and CyA blood concentrations increased after a diarrheal episode due to a bacterial or viral infection [38–41]. Despite a probable effect of diarrhea on absorption, the authors concluded that intestinal inflammation suppressed the activity of CYP3A [38]. Similarly, in adults, several studies and case reports have focused on the impact of hepatitis C infection on tacrolimus and CyA

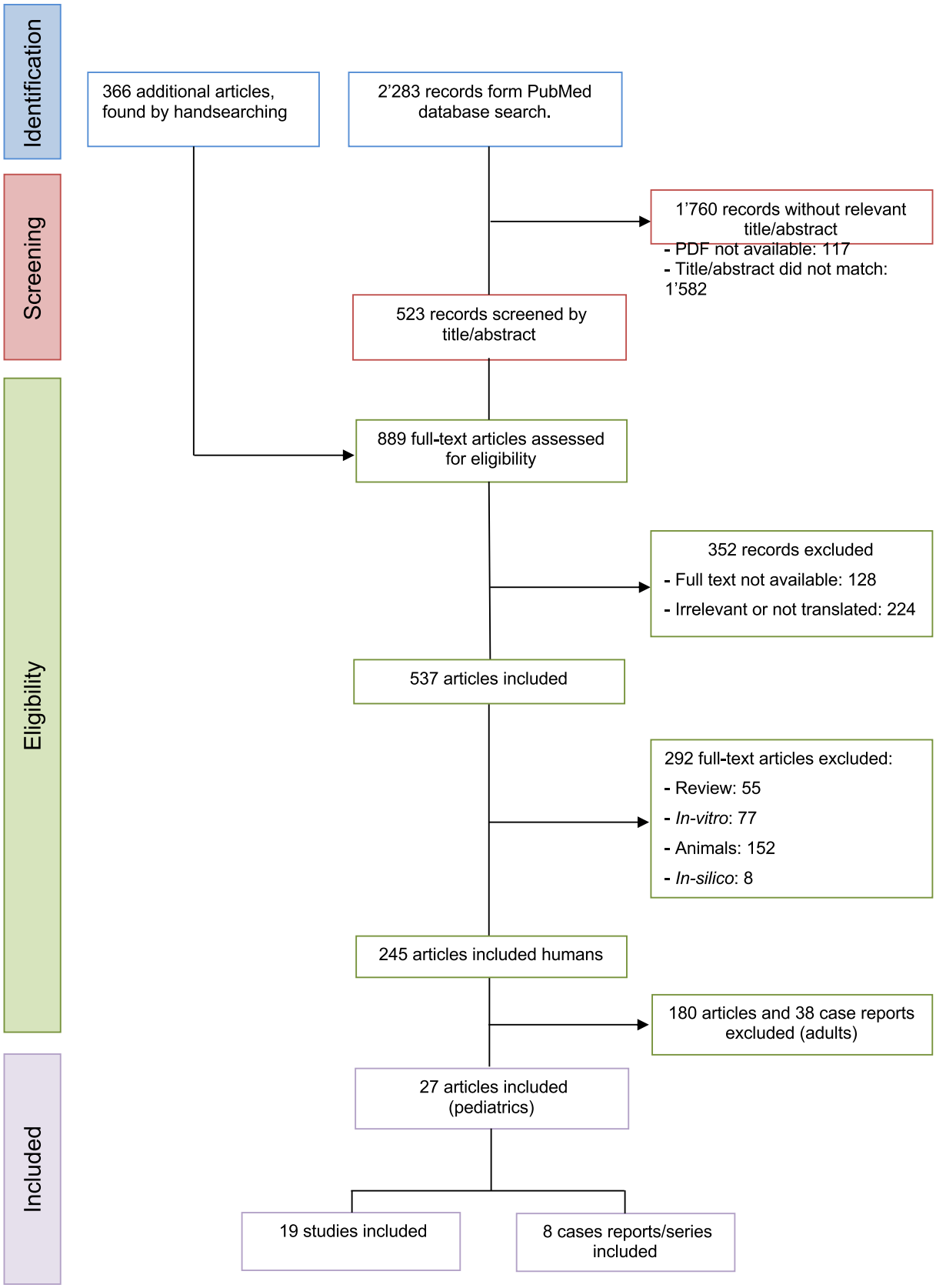

Fig. 1 Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) flowchart of the studies selection process

Table 1 Impact of difference sources of inflammation on CYPs activities

Inflammation characterized by	Victim drugs (CYPs concerned)	Age and number of subjects	Potential effect of interaction	Relevant comments	References and design
Upper respiratory tract infection	Theophylline (CYP1A2)	9–15 years ($n = 10$)	Mean plasma half-life was significantly longer during serologically proven infection compared with 1 month after illness (419.8 vs 249.9 min, p = not shown) [$n = 6$] Plasma half-life did not change during febrile illness without seroconversion ($p > 0.1$) [$n = 4$]	Transaminases were in the normal range and creatinine clearance did not change. No CYP modulators were introduced but there was no mention of potential usual treatment	[25] Cohort study
Bronchiolitis	Theophylline (CYP1A2)	3 weeks to 6.5 months ($n = 12$)	Mean clearance was lower in children with infection than previously published in patients of comparable age without any viral infection (p = not shown)	One child had cystic fibrosis and one had gastroesophageal reflux. No concomitant use of a CYP1A2 modulator	[26] Cohort study
Respiratory syncytial virus infection	Theophylline (CYP1A2)	6–48 months ($n = 29$ infection and 29 controls)	Clearance was not significantly different between both groups (1.32 ± 0.14 and 1.25 ± 0.05 mL/kg/min, respectively)	No mention of concomitant treatment, or even organ laboratory values to monitor organ dysfunction	[27] Case-control study
Flu-like symptoms	Theophylline (CYP1A2)	Age between 3 and 11.5 years ($n = 11$)	Clearance was reduced compared with previous determination of steady-state concentrations, but this reduction was not significant (p = not shown), but ten children had symptoms of theophylline toxicity Six had an influenza B titer and four were negative for serologic findings, and the mean difference in serum theophylline concentration between pre-infection and post-infection was 20 µg/mL and 14.2 µg/mL, respectively, for positive patients but this was not significant	Exclusion criteria were: increased dosage of theophylline, use of concomitant antibiotics, and symptoms of flu-like illness within the prior 2 weeks	[28] Cohort study
Febrile illness	Theophylline (CYP1A2)	11 and 7 years ($n = 2$, sex unknown)	Elevated serum theophylline concentrations (> 20 µg/mL) after febrile illness, adverse drug effects characteristic of inappropriate theophylline dosing	–	[28] Case report

Table 1 (continued)

Inflammation characterized by	Victim drugs (CYPs concerned)	Age and number of subjects	Potential effect of interaction	Relevant comments	References and design
Respiratory syncytial virus infection	Theophylline (CYP1A2)	3–6 months ($n = 3$, sex unknown)	Clearance was lower in the seropositive children as compared with the other 2 (p = not shown)	–	[29] Case report
Influenza vaccination	Theophylline (CYP1A2)	15 years ($n = 1$, female)	The patient was usually known to metabolize theophylline rapidly Levels increased to a peak 5 h after vaccination and slowly returned to normal levels over the next 24 h	No CYP modulators	[30] Case report
Acute asthma exacerbation	Aminophylline (CYP1A2)	1–15 years ($n = 52$)	Patients with lower C72h/C24h ratios had a significantly higher number of patients with a CRP level > 0.5 mg/dL or fever > 37.5 °C Patients with a lower ratio had reduced CYP1A2 activity on admission because of higher CRP and fever levels and their catabolizing capacity improved during treatment	No other medication that affects theophylline metabolism such as anticonvulsants, rifampin, macrolide, or quinolone antibiotics was administered within the week prior to and during the study	[31] Cohort study
Malaria	Caffeine (CYP1A2)	7–9.9 years ($n = 5$ controls) 3–9 years ($n = 5$ malaria)	$t_{1/2}$ and oral clearance of caffeine were respectively longer and lower in children with malaria than in healthy volunteers (9.2 ± 3.5 h vs 3.7 ± 1.8 h, $p < 0.01$ and 1.6 ± 1.0 vs 4.4 ± 1.9 mL/min/kg, $p < 0.05$) Metabolic ratio was five to ten times lower in children suffering from malaria than in controls of the various timepoints	Four of five children with the diagnosis of malaria were treated with chloroquine and one was receiving artemether. There were no CYP1A2 modulators	[32] Case-control study

Table 1 (continued)

Inflammation characterized by	Victim drugs (CYPs concerned)	Age and number of subjects	Potential effect of interaction	Relevant comments	References and design
Intensive care unit	Midazolam (CYP3A)	2 days to 17 years ($n = 18$ intensive care) 3–10 years ($n = 56$ controls)	Clearance and elimination $t_{1/2}$ determined during and after continuous infusion in intensive care patients were 5.0 ± 3.9 mL/kg/min and 5.5 ± 3.5 h, respectively Total body clearance and plasma elimination $t_{1/2}$ in controls were 9.11 mL/kg/min and 1.17 h, respectively	None of the patients received midazolam > 12 h before study or treatment that altered the PK of midazolam, but 2 patients received such a drug after inclusion. Three patients were considered as outliers (severe renal and hepatic failure and erythromycin intake). Pharmacokinetic variations could also be explained by variations in body composition (volume of distribution)	[33] and [34] Case-control study
Critically ill children	Midazolam (CYP3A)	2 days to 17 years ($n = 21$)	Clearance was significantly lower in children with multiple organ failure ($p = 0.035$) than in those without No correlation was found between CRP and clearance ($r = -0.27$, $p = 0.30$) No correlation between clearance corrected for body weight and the administered dose ($r = -0.41$, $p = 0.06$)	It was a pilot study. No mention of concomitant treatment or laboratory values. Alternative explanation could be the altered level of protein binding. Inflammation may alter drug PK and PD differently as decreased clearance is seemingly unrelated to decreased dose requirements	[35] Cohort study
Intensive care unit	Midazolam (CYP3A)	1 day to 7 years [median = 5.1 months] ($n = 83$)	Higher CRP concentration was associated with lower clearance CRP of 300 mg/L was associated with a 65.4% lower clearance than a CRP of 10 mg/L	The clearance of midazolam decreased with an increasing number of organ failures. 14 patients received a CYP3A inhibitor, but this had no effect on midazolam clearance. CYP3A polymorphisms, albumin, creatinine, and alanine aminotransferase levels were tested as covariates, but neither improved the model nor explained variability in clearance or volume of distribution. Inflammation and organ failure resulted in a better description of the data	[36] Cohort study

Table 1 (continued)

Inflammation characterized by	Victim drugs (CYPs concerned)	Age and number of subjects	Potential effect of interaction	Relevant comments	References and design
Acute lymphoblastic leukemia	Lorazepam (CYP3A)	Mean age = 5.3 years (n = 14)	Mean increase of 52% (p = 0.016) in systemic clearance between the pre-induction and the post-induction therapy phase All patients were in remission in the post-induction therapy phase, and an effect of induction therapy was not expected, as clearance was measured 46 (35–96) days after	Clinical factors such as total bilirubin, SGOT, PT time, WBC count at diagnosis, age, and liver size at diagnosis were not predictive for the changes seen in model substrate clearance before and after induction. No influence of fever, induction therapy, or concurrent drug therapy. Changes in protein binding cannot account for the improvement in clearance of lorazepam, as lorazepam free clearance increased before and after remission	[37] Cohort study
Diarrheal episode caused by bacterial infection	Cyclosporin A (CYP3A4)	1.8 years (n = 1, boy)	Elevated C/D ratio from 2.0 to 2.9 to 6.3 during a diarrheal episode Increased C/D ratio to 6.3, then dropped below 4 after diarrhea remission Inflammation in the intestine caused by bacterial infections suppressed the activity of CYP3A and led to an increase of the C/D ratio of CyA	No CYP modulators and no change in laboratory values	[38] Case report
Diarrheal episode caused by rotavirus	Tacrolimus (CYP3A4)	2.4 years (n = 1, girl)	C/D ratio elevated from 5.4 to 5.7 to 11.3 during a diarrheal episode Upon remission of diarrhea, tacrolimus concentration decreased Inflammation of the intestine caused by viral infections suppressed the activity of CYP3A and led to an increase of the C/D ratio of tacrolimus	No CYP modulators and no change in laboratory values	[38] Case report

Table 1 (continued)

Inflammation characterized by	Victim drugs (CYPs concerned)	Age and number of subjects	Potential effect of interaction	Relevant comments	References and design
Diarrheal episode caused by rotavirus	Tacrolimus (CYP3A4)	Age and sex unknown ($n = 1$)	Increase in tacrolimus trough concentration from 9 ± 1.5 to 60 µg/L (despite drug withdrawal) after diarrheal episodes	No CYP modulators, weight loss, or hepatic dysfunction. Decreased GI transit time might be a potential mechanism for increasing tacrolimus concentrations. The expression on the small and large intestinal epithelium of P-gp could be of clinical importance. The destruction of villous epithelial cells may be an important determinant	[39] Case report
Diarrheal episode caused by rotavirus	Tacrolimus (CYP3A4)	7 years ($n = 1$, boy)	Increase in tacrolimus trough concentration from 9.5 ± 0.7 to 20.9 µg/L, despite tapering the dose, after a diarrheal episode	No CYP modulators, weight loss, or hepatic dysfunction. Decreased GI transit time might be a potential mechanism for increasing tacrolimus concentrations. The expression, on the small and large intestinal epithelium of P-gp could be of clinical importance. The destruction of villous epithelial cells may be also an important determinant	[39] Case report
Gastroenteritis	Tacrolimus (CYP3A4)	9 years ($n = 1$, girl)	Trough concentration was higher than usual at 27.6 ng/mL	Authors suggest that it is the combined effect of altered gut motility and hepatic metabolism. Laboratory values were in the normal limits. No change in concomitant treatment but loss of weight (no change in diet)	[40] Case report

Table 1 (continued)

Inflammation characterized by	Victim drugs (CYPs concerned)	Age and number of subjects	Potential effect of interaction	Relevant comments	References and design
Shigella infection	Tacrolimus (CYP3A4)	8 years ($n = 1$, girl)	Usual blood concentrations was 8.2 mg/mL, but it increased to more than 30 ng/mL on admission because of fever (39–40 °C), diarrhea, and abdominal cramps that had started a week earlier Over the next 2 weeks, tacrolimus blood concentrations ranged between 16.5 and 22.0 ng/mL despite reductions in tacrolimus dose After the diarrhea resolved, tacrolimus blood concentration returned to baseline	Liver function was stable, but Shigella infection facilitates the invasion, rupture, and permeability of the intestinal epithelium. No dehydration or diet changes	[41] Case report
Flu-like symptoms	Sirolimus (CYP3A)	8 and 13 years ($n = 2$ boys)	Higher than expected concentrations were observed in patients with flu-like symptoms and, therefore, an infectious state with fever	No CYP modulators during treatment and time lapse between the onset of fever and decrease in clearance	[42] Case report
Treatment with basiliximab	Cyclosporin (CYP3A)	Mean age = 7.5 years ($n = 24$ basiliximab) Mean age = 9.7 years ($n = 15$ controls)	Dose required during the first 10 days was lower in the basiliximab group than in controls, while the trough concentration was higher At days 28–50, the concentration decreased despite any change in dose	–	[43] Case-control study
Unspecified source of inflammation (150 < CRP > 150 mg/L)	Voriconazole (CYP3A4 and CYP2C19)	< 12 years [median = 4 years] ($n = 11$) > 12 years [median = 15 years] ($n = 16$)	All groups received the same doses, based on mg/kg body weight Patients aged older than 12 years with CRP levels > 150 mg/L had significantly higher trough concentrations of voriconazole CRP > 150 mg/L downregulated CYP2C19 and 3A4 in children aged > 12 years	Exclusion criteria were concomitant use of CYP modulators and relatively low/high dosage to avoid bias due to extreme dosing. Patients' characteristics (underlying disease, trough concentration, and CRP value) were similar between both groups	[44] Cohort study

Table 1 (continued)

Inflammation characterized by	Victim drugs (CYPs concerned)	Age and number of subjects	Potential effect of interaction	Relevant comments	References and design
Aspergilloses	Voriconazole (CYP2C19 and 3A)	9 months to 18 years ($n = 10$)	8 patients had hematological malignancy and 2 had cystic fibrosis Median trough concentrations in patients < and > than 12 years were 0.53 and 0.79 mg/L, respectively CRP had no significant impact on trough concentrations of voriconazole	Impact of age, sex, weight, survival, route of administration, co-treatment (omeprazole, phenytoin, and CyA), registered biochemical parameters, and total daily dose on voriconazole trough concentrations was examined. None of these factors had a significant impact ($p > 0.5$)	[45] Cohort study
Hepatitis A	Coumarin (CYP2A6)	6–10 years ($n = 11$ hepatitis A) 6–13 years ($n = 10$ controls)	Mean reduction of 72% ($p < 0.0001$) in total urine excretion of 7-hydroxycoumarin compared with healthy controls	ASAT, ALAT, and GGT were below normal range in patients with hepatitis A. Creatinine was in normal range. Unknown concomitant treatments	[46] Case-control study
Gastroenteritis	Oxatomide (CYP2D6 and 3A4)	3 years ($n = 1$, boy)	Anti-H_1 toxicity (abdominal pain, pallor, slurred speech followed by serious long-lasting impairment of consciousness) after oxatomide, despite not having any of the following CYPs polymorphisms: CYP2D6*3, *4, *5, and *6 or CYP3A4*1B CRP value of 0.47 mg/dL (physiologic range < 0.25 mg/dL) was found	No other drug treatment and no history of recent trauma, seizure, and neurologic disorder	[47] Case reports
Febrile illness	Anticonvulsants (CYP1A2, 2C9, 2C19, 2E1, and 3A)[a]	6 months to 7 years [mean = 10 years] ($n = 111$)	55 episodes of febrile illness in 39 children during the study period 12 illnesses were associated with significant increases or decreases in serum levels 7 children experienced toxic clinical symptoms and one had increased seizures during illness	27/55 and 49/55 were treated with antibiotics and acetaminophen, respectively. Authors conclude that mechanisms of anticonvulsant level changes appeared to include interaction with antibiotics, antipyretics, or viral illness. However, investigators may have missed some illnesses	[48] Cohort study

Table 1 (continued)

Inflammation characterized by	Victim drugs (CYPs concerned)	Age and number of subjects	Potential effect of interaction	Relevant comments	References and design
Fever	Antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18, and 3A4)[a]	5 months to 5 years ($n = 6$)	The saliva clearance of antipyrine was reduced by approximatively 50% during fever compared with the afebrile period ($p < 0.02$) The half-life during fever was almost doubled ($p < 0.01$)	Concomitant treatments (erythromycin in case 2 and cotrimoxazole in case 3) but the clearance was reduced during fever in all children by 8–64%	[49] Cohort study
Suspected sepsis	Antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18, and 3A4)[a]	1–18 years [median = 4 years] ($n = 51$ suspected sepsis and 6 controls)	Metabolism was lower in the children with suspected sepsis than in the 6 children in the control group Metabolism was much lower in patients with multiple organ failure, and the antipyrine elimination half-life increased with increasing IL-6 and nitrate plus nitrite levels	Patients were assigned 1 point for each organ failure. Univariate analysis revealed an association between reduced antipyrine metabolism and liver, respiratory, and hematological failure ($p < 0.05$). Patients were excluded if they received any exogenous NO source. Concomitant use of CYP modulators was recorded. There were 38 positive cultures	[50] Case-control study
Acute lymphoblastic leukemia	Antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18, and 3A4)[a]	Mean age = 5.3 years ($n = 14$)	Mean increase of 67% ($p = 0.007$) in systemic clearance between the pre-induction and the post-induction therapy phase All patients were in remission in the post-induction therapy phase, and an effect of induction therapy was not expected, as clearance was measured 46 (35–96) days after	Clinical factors such as total bilirubin, SGOT, PT time, and WBC count at diagnosis, age, and liver size at diagnosis were not predictive for the changes seen in model substrate clearance before and after induction. No influence of fever, induction therapy received, or concurrent drug therapy	[37] Cohort study
Crohn's disease	–	7–15 years ($n = 19$ Crohn's disease and 19 controls)	Higher CYP3A4 and CYP3A5 expression levels detected in Crohn's disease biopsies compared with normal biopsies Crohn's disease group biopsies came from non-inflamed duodenal biopsies	All included patients were not receiving known modulators of CYP3A and had no digestive complications, but a tissue expression discrepancy should be taken into consideration	[51] Case-control study

Table 1 (continued)

Inflammation characterized by	Victim drugs (CYPs concerned)	Age and number of subjects	Potential effect of interaction	Relevant comments	References and design
Crohn's disease	–	7–17 years ($n = 18$ Crohn's disease and 12 controls)	PXR expression was decreased in the inflamed terminal ileum compared with the non-inflamed duodenum ($p < 0.001$) but this was not observed in the control group ($p = 0.52$) CYP3A4 expression followed the same line ($p = 0.014$ and $p = 0.61$, respectively) Expression of PXR/CYP3A4 was inversely correlated with IL-8 and inflamed tissue	Use of non-inflamed duodenal tissue from each subject as a negative control for that subject eliminates inter-individual genetic variability as a confounding factor. No difference in PXR expression was observed between the terminal ileus and the duodenum in age-matched and sex-matched controls, the observed decrease in the CD terminal ileus cannot be attributed to the biopsy-site location	[52] Case-control study

ALAT alanine aminotransferase, *ASAT* aspartate aminotransferase, *C/D* concentration/dose, *CRP* C-reactive protein, *CyA* cyclosporin A, *CYP* cytochrome P450s, *GI* gastrointestinal, *GGT* gamma-glutamyl transferase, *h* hours, *IL* interleukin, *min* minutes, *NO* nitric oxide, *PD* pharmacodynamics, *P-gp* P-glycoprotein, *PK* pharmacokinetics, *PT* Prothrombin Time, *SGOT* Serum Glutamic-Oxaloacetic Transaminase, $t_{1/2}$ half-life, *WBC* white blood cell

[a]Definitive conclusion cannot be drawn because of the number of CYP isoforms that contribute to the metabolism of the drug

pharmacokinetic parameters. Indeed, the plasma concentrations of tacrolimus and CyA were higher, and doses lower, in patients with hepatitis C infection as compared with patients without hepatitis C infection [53–55]. Inversely, treatment of hepatitis C resulted in decreased tacrolimus and CyA concentrations and/or increased required doses [56–60]. Thus, the treatment of the infection allowed a return to baseline CYP3A activity, probably because the subsequent inflammation disappeared.

Basiliximab is another example where inflammation downregulated CYP3A activity similarly in children and adults. Indeed, concentrations of tacrolimus and CyA increased during the first days of basiliximab treatment both in adults and children [43, 61]. Moreover, concentrations decreased spontaneously after 30 days of basiliximab treatment, despite any dose modification [43, 61]. The authors suggested that the impact of basiliximab on drug metabolism was due to interleukin-2 [61]. The similar effects observed in adults and children could be explained by the intermediate developmental pattern of CYP3A4, as adult CYP3A activity is reached at the end of infancy and children were aged older than 2 years [17–19].

Regarding critically ill patients, CYP3A4 has been shown to be downregulated in adults and the decrease of CYP34 activity was correlated with the severity of organ failure [62, 63]. The same results were observed in pediatric intensive care unit patients. Metabolism of midazolam decreased with the severity of intensive care unit-induced inflammation as a consequence of CYP3A4 downregulation [33–36]. However, it is possible that mechanisms other than CYP regulation could also be responsible for the drug's pharmacokinetic alterations during inflammatory states, such as changes in plasma protein binding and renal excretion [64]. A study indeed showed that proinflammatory cytokines trigger an acute-phase response that could increase the unbound fraction of drugs [65]. In diabetic adults, the lack of differences in CyA daily doses, but the lower production of its metabolites, could be the consequences of variations in protein binding [66]. No other studies assessing CYPs other than CYP3A could be found in the literature. Those studies should be performed, as data in adults have shown that CYPs are regulated in an isoform-specific manner in critically ill patients [67].

In children with Crohn's disease, both CYP3A4 and CYP3A5 were upregulated, which is inconsistent with the previous observation of downregulation of CYP3A4 associated with higher C-reactive protein (CRP) levels, but could be explained by the fact that the biopsies were from non-inflamed tissue [51]. In another study in children of the same age range, the expression of the nuclear hormone receptor PXR was decreased in inflamed tissues and, thus, CYP3A4 expression also decreased [52]. Further studies in children with inflammatory bowel disease should be initiated to

understand these discrepancies, as well as in vitro studies to understand the underlying mechanisms. In adults, verapamil (CYP3A4, 1A2, 2C8, 2C9, and 2C18 substrate) and propranolol (CYP2D6 substrate) concentrations were significantly higher in patients with active Crohn's disease than in healthy volunteers or patients in remission [68, 69]. The authors suggested that the reduced clearance could be attributed to CYP downregulation, but increased bioavailability due to downregulation of P-glycoprotein could not be ruled out [68]. However, a possible impact of the decrease in CYP content due to Crohn's disease should be kept in mind.

4.2 CYP1A2

In adults, the impact of inflammation on CYP1A2 activity has been well studied for two substrates (i.e., theophylline and clozapine) and a decrease in their clearances has been observed, as well as symptoms of clozapine toxicity [70–78]. In pediatrics, theophylline was the only studied substrate of CYP1A2, except one study with caffeine [32]. Theophylline has been a commonly used drug for asthma for over 50 years, but its narrow therapeutic index has made it disappear from current asthma guidelines [79, 80].

In line with what is found in adults, our literature review showed that infection may decrease CYP1A2 activity in children [25, 26, 28–30, 32]. However, a study conducted in children aged 6–48 months ($n = 58$), showed that infection had no impact on theophylline clearance [27]. CYP1A2 has the slowest developmental pattern and a large heterogeneity in the impact of inflammation on its activity is expected, with increasing intensity as age advances [17–19]. Further investigations are needed to determine whether CYP1A2 is affected by inflammation in children.

4.3 Other CYPs

Antipyrine is an older drug metabolized by several CYPs (CYP1A2, 2B6, 2C8, 2C9, 2C18, and 3A4) and which has been widely used to investigate hepatic drug metabolism because it is almost completely absorbed from the intestine, has negligible plasma protein binding, a low hepatic extraction ratio, and is metabolized almost entirely by the liver [81]. In children, clearance of antipyrine appeared to be reduced during fever or suspected sepsis [49, 50]. Moreover, the inhibition of metabolism was proportional to disease severity and interleukin-6 levels, and a return to baseline levels was observed with cancer resolution [37, 50]. Inflammation is present at all stages of cancer, with an apparent link between certain immune-mediated diseases or infection and cancer, such as inflammatory bowel disease or *Helicobacter pylori* that are associated with colorectal and gastric cancer, respectively [82]. CYP3A and CYP2C19 are two well-studied isoforms in cancer, and several studies have found impaired activity of these CYPs in adult patients with cancer [83–90]. Only one study has been conducted in children (mean age 5.3 years) with acute lymphoblastic leukemia, and CYPs also appear to be altered during the acute phase [37]. Further studies are needed in pediatric oncology and in different age groups because chemotherapeutic and antimicrobial agents for prophylaxis are CYP substrates. In adults, the downregulation of antipyrine metabolism was also observed during infection, diabetes, or interferon treatment [91–94].

Anticonvulsants studied in children during inflammation were carbamazepine (CYP1A2, CYP2C9, and CYP3A substrate), phenytoin (CYP2C9 and CYP2C19), valproate (CYP2C9), phenobarbital (CYP2C9 and CYP2C19), and ethosuximide (CYP2E1 and CYP3A) [48]. Only seven children out of 39 with a febrile illness experienced toxic clinical symptoms and one had increased seizures during the illness [48]. The authors conclude that nearly a quarter of patients with febrile illnesses experienced significant changes in drug concentrations, with 9% developing clinical toxicity [48]. They suspected direct inhibition by antibiotics and plasma protein displacement by antibiotics and antipyretics, in addition to inhibition of CYP activity by viral infection [48]. In adults, the effect of inflammation was mostly studied with phenytoin with an increased risk of toxicity. For instance, a 52-year-old woman had toxic phenytoin concentrations with associated symptoms during the influenza illness and urinary excretion of the metabolite of mephenytoin among patients with liver disease or multiply injured was significantly lower than healthy controls [67, 95, 96].

Voriconazole is mainly metabolized by CYP2C19 and CYP3A [97]. In adults, a cohort study found that the level of CRP was positively associated with the concentration/dose (C/D) ratio or through concentration of voriconazole and was negatively associated with the metabolic ratio expressed by [N-oxide voriconazole]/[voriconazole] [98–101]. Moreover, an elevated level of CRP was a risk factor for voriconazole overdose [102]. This could be explained by CYP2C19 and/or CYP3A downregulation due to inflammation, represented by elevated levels of CRP. In children, this association is less pronounced, as a significant association between CRP levels >150 mg/L and higher voriconazole through concentrations was only observed in patients aged older than 12 years [44]. Moreover, another study conducted in children did not find an association between trough concentrations of voriconazole and CRP, but the cohort was very small and no distinction was made between older and younger children [45]. Possible explanations for this difference in association between CRP and CYP downregulation observed in children aged younger and older than 12 years are that the PK of voriconazole appears to be linear before 12 years of age and non-linear after [44]. The bioavailability of voriconazole indeed decreases (and clearance increases) in pediatric

patients compared with adults, resulting in less saturation of PK processes and, thus, linear kinetics [12, 45]. This implies that first-pass metabolism is higher in the pediatric population and it was suggested that CYP-mediated clearance is higher in children under 12 years of age and thus that downregulation by inflammation had less impact on voriconazole metabolism [44]. However, CYP2C19 and CYP3A4 activity reaches that of an adult at the end of infancy and inflammation is expected to further inhibit CYP activity because there are more CYPs to downregulate [17–19]. One possible explanation is that enzymes other than CYPs are responsible for voriconazole clearance and that they are more expressed in children and less impacted by inflammation. Voriconazole is also metabolized by flavin-containing monooxygenase 3, and the contribution of flavin-containing monooxygenase 3 and CYP2C19 has been shown to be five-fold and three-fold higher in children than in adults, respectively [45, 103]. It is important to consider these non-CYP phase I drug-metabolizing enzymes because approximatively 25% of metabolically eliminated drugs are first subjected to non-CYP-mediated biotransformation [14]. Moreover, it seems that flavin-containing monooxygenase 3 and CYP2C19 have higher catalytic activity in children than in adults [45, 103]. Another hypothesis is that the CYP2C19 contribution to CYP3A is more affected by inflammation. We have indeed previously demonstrated in adults that CYP3A was more impacted by inflammation than CYP2C19 [104].

4.4 CYP Genotype

The CYP genotype is an additional factor to be considered, as cytokines may not have the same impact on CYP activities depending on the basal genetic activity of CYPs. In adults, the different impact of inflammation on CYP2C19 and CYP2C9 depending on the genotypes has already been demonstrated [105, 106].

In children, the same conclusions can be drawn as oxotamide toxicity was observed in 3-year-old children who were not carriers of CYP2D6 or CYP3A4 main allelic variants at the time of the study, meaning that the reduced clearance is not caused by the manifestation of CYP2D6*3, *4, *5, and *6 or CYP3A*1B [47]. It is therefore conceivable that the release of pro-inflammatory molecules such as CRP decreased CYP2D6 and/or CYP3A activities, eventually leading to an increase in the oxatomide plasmatic concentration [47].

4.5 Limitations

Our systematic review has some limitations, which suggest a cautious approach to these results. First, the manual search was performed in a single database and for published articles only, which cannot rule out publication bias and the potential for omissions. Furthermore, the studies found and selected were poorly comparable to each other, owing to the heterogeneity of their overall methodology. Finally, the observed PK and clinical impact are neither robust nor generalizable because of the diversity of the sources and severity of inflammation.

5 Conclusions

In recent years, numerous clinical studies and case reports evaluated in adults have reported a modification in CYP activities and pharmacokinetic parameters of drugs in the presence of inflammation. The latter being an important factor contributing to variation in CYP activities between and within individuals. This may have a clinical impact, as CYPs play an essential role in the bioactivation or elimination of many therapeutic agents. Current data suggest that inflammation has an isoform-specific and intensity-specific impact, i.e., CYP3A and CYP2C19 activities are downregulated and CYP2D6 activity does not change during inflammation, whereas the impact on CYP1A2, CYP2B6, and CYP2C9 remains unclear and needs further investigations. Some studies have even shown that CYP activity returns to baseline after the improvement of the disease. There is significant heterogeneity in inflammatory markers, depending on the disease involved and its degree of severity.

To our knowledge, no study has evaluated inflammation-induced CYP phenoconversion in children using a cocktail approach. In addition to the moral, ethical, and legal difficulties of conducting studies in children, a cocktail approach is further complicated by the multitude of probes administered. The development of endogenous markers of CYP metabolism could help overcome these obstacles and may have interesting opportunities to develop personalized medicine in the pediatric field. Indeed, in children, the proportion of the drug cleared by the metabolism of CYPs, the patient's genotype, and concomitant medications, but also ontogeny must also be taken into account. Inflammation has a different impact on CYP activity depending on age as the proportion of CYP changes depending on the isoform and extrapolation from adult data cannot be done automatically. Despite all this evidence, much remains to be done to know the impact of inflammation on CYPs activity in the pediatric population. Indeed, this review highlights that, beyond the fact that few studies have been conducted in pediatrics, almost no studies have been conducted in neonatal to early infancy, although this is the period when developmental changes are most important. Moreover, many diseases with underlying inflammation have not yet been studied and the few existing studies do not focus on CYPs other than CYP3A and CYP1A2.

Declarations

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Conflict of Interest/Competing Interests The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

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Availability of Data and Material The data that support the findings of this study are available from all contributing authors and upon reasonable request.

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Authors' Contributions CL participated in the manuscript conceptualization, experimental design, writing, data analysis, and overall manuscript review. FR, CFS, and VR participated in the manuscript conceptualization, experimental design, data analysis, supervision, and overall manuscript review. JAD participated in the supervision and overall manuscript review.

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Chapter 8: Prediction of cytochromes P450 3A and 2C19 modulation by both inflammation and drug interactions using physiologically based pharmacokinetics.

Summary

As discussed in **chapter 1**, PBPK modeling can be used to predict potential clinical DDIs and PK profile of drugs in special populations, among other applications. These models use the « bottom-up » approach and are based on several input parameters divided into three categories, namely the system (physiological properties), the drug (physicochemical and ADME characteristics) and the study design. With a quantitative mechanistic framework, PBPK models are gradually becoming important for the prediction of DDIs, ADRs and drug-disease interaction.
For example, **research article 4** in **chapter 8** attempted to simultaneously model the impact of a DDI and a drug-disease interaction on CYP3A and CYP2C19 activity to support precision medicine. Indeed, **chapter 2 to 3** and **chapter 4 to 7** demonstrated that CYPs activity is significantly influenced by DDIs and drug-disease interaction, respectively, which may lead to safety and efficacy concerns.

The **research article 4** was published in another journal of the *Clinical Pharmacology and Therapeutics* family, namely *Pharmacometrics and Systems Pharmacology*. The aim was to build PBPK models that would predict the *in vivo* data from **research article 2** regarding CYP3A and CYP2C19 activity. Inflammatory state (defined by IL-6) was triggered during surgery and esomeprazole was prescribed during the first post-operative days to prevent the occurrence of stress ulcer. Esomeprazole is a well-known CYP2C19 inhibitor, leading to DDIs, and as shown in **chapter 6**, IL-6 is known to reduce CYP3A and CYP2C19 expression and activity.
In the **research article 4**, *in vitro* and animal models were used to construct the models for CYP3A and CYP2C19 probe substrates (midazolam and omeprazole) and their main metabolite (victim drugs), as well as molecules responsible for inhibition of their metabolism (perpetrators). Midazolam, 1-OH-midazolam, omeprazole, 5-OH-omeprazole, esomeprazole and IL-6 models were thus built. The models were validated with data from the literature and a correction factor was applied to convert drug concentrations from whole blood (DBS) to plasma. The clinical data of **research article 2** allowed to demonstrate that the impact of IL-6 (drug-disease interaction) and esomeprazole (DDI) on CYP3A and CYP2C19 activity after hip surgery were correctly predicted with the developed models.

My contributions to this **research article 4** were research design, data analysis, models development, contribution of analytical tools to perform the research and writing the article.

Research article 4: Prediction of cytochromes P450 3A and 2C19 modulation by both inflammation and drug interactions using physiologically based pharmacokinetics

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ARTICLE

Prediction of cytochromes P450 3A and 2C19 modulation by both inflammation and drug interactions using physiologically based pharmacokinetics

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Abstract

Xenobiotics can interact with cytochromes P450 (CYPs), resulting in drug–drug interactions, but CYPs can also contribute to drug–disease interactions, especially in the case of inflammation, which downregulates CYP activities through pretranscriptional and posttranscriptional mechanisms. Interleukin-6 (IL-6), a key proinflammatory cytokine, is mainly responsible for this effect. The aim of our study was to develop a physiologically based pharmacokinetic (PBPK) model to foresee the impact of elevated IL-6 levels in combination with drug interactions with esomeprazole on CYP3A and CYP2C19. Data from a cohort of elective hip surgery patients whose CYP3A and CYP2C19 activities were measured before and after surgery were used to validate the accurate prediction of the developed models. Successive steps were to fit models for IL-6, esomeprazole, and omeprazole and its metabolite from the literature and to validate them. The models for midazolam and its metabolite were obtained from the literature. When appropriate, a correction factor was applied to convert drug concentrations from whole blood to plasma. Mean ratios between simulated and observed areas under the curve for omeprazole/5-hydroxy omeprazole, esomeprazole, and IL-6 were 1.53, 1.06, and 0.69, respectively, indicating an accurate prediction of the developed models. The impact of IL-6 and esomeprazole on the exposure to CYP3A and CYP2C19 probe substrates and respective metabolites were correctly predicted. Indeed, the ratio between predicted and observed mean concentrations were <2 for all observations (ranging from 0.51 to 1.7). The impact of IL-6 and esomeprazole on CYP3A and CYP2C19 activities after a hip surgery were correctly predicted with the developed PBPK models.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

There is high interindividual variability in cytochrome P450 (CYP) activities due to genetic, environmental, and physiological factors, including drug–drug and

drug–disease interactions. The development and use of a physiologically based pharmacokinetic (PBPK) model allow for the prediction of the pharmacokinetic properties of a drug and have been used to predict and assess drug efficacy and safety.

WHAT QUESTION DID THIS STUDY ADDRESS?

Did the PBPK models developed accurately predict the impact of elevated interleukin-6 (IL-6; acute inflammation) in combination with drug interactions with esomeprazole on CYP3A and CYP2C19 activities?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The impact of IL-6 and esomeprazole on exposure to the CYP3A and CYP2C19 probe substrates and their respective metabolites were correctly predicted by the developed PBPK models.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

The integration and prediction of pharmacodynamic and disease parameters in PBPK models appear to be a promising approach to personalize treatments.

INTRODUCTION

The interplay of genetic, physiological, and environmental factors leads to interindividual and intraindividual variability in response to treatment.[1] Indeed, these factors are unique to each individual and/or may change over time and cause disparity in the safety and efficacy of treatments.[1] Precision medicine is a leading approach in the transformation of medicine, aiming to tailor treatments according to the biological and genetic characteristics of individuals.[2] By using the "five rights of medication administration," personalized medicine supports optimization of therapy in terms of efficacy and safety and thus public health and healthcare costs.[3] In fact, one size does not fit all, and 40%–70% of patients have a lack of efficacy or safety in their pharmacological therapy.[4]

The causes of variability in drug responses and the interaction between the body and the drug must be better considered to personalize medicine.[5] Cytochromes P450 (CYPs) are the major enzymes involved in drug metabolism and responsible for about three-quarters of drugs cleared by metabolism.[6] It is estimated that 15%–30% of the variability in their activities is caused by genetic polymorphisms, but other nongenetic factors may also greatly contribute to this observed variability.[4,7] Xenobiotics and endogenous substances may inhibit or induce CYP activity such as the well-known modulation of CYP activities by certain concomitant treatments, resulting in pharmacokinetic (PK) drug–drug interactions (DDIs).[8] In vitro and animal model data as well as smaller human data support the theory that inflammation downregulates CYP activities, resulting in a PK drug–disease interaction.[9]

Inflammation is a complex biological protective response to stimuli such as pathogens, damaged cells, or irritants.[10] It involves a large repertoire of host cells, blood vessels, proteins, and numerous mediators to eliminate the initial cause and launch the healing process.[10] Immune cells are activated by the pattern-recognition receptors to trigger the inflammatory response and are considered to be the main source of various proinflammatory mediators, such as cytokines.[10] Main proinflammatory cytokines are interleukin (IL)-1β, IL-6, and tumor necrosis factor-α, and they are deemed as the most important mediators of acute phase protein synthesis in hepatocytes.[10,11] IL-6 is a critical cytokine that mediates many inflammatory and immunomodulatory pathways against a multitude of environmental and infectious stimuli.[12] IL-6 levels increase to a maximum between 4 and 48 h after surgery and drop rapidly after 48 to 72 h.[13]

The mechanism of modulation of CYP activities by inflammation is complex and includes a wide variety of ligand-activated transcription factors and mediators, but the cytokine-mediated alteration of gene transcription is the major mechanism of modulation.[7] The downregulation of CYP during the inflammatory response can occur through transcriptional downregulation of transcription factors, interference with dimerization/translocation of transcription factors, alteration of liver-enriched CCAATT/enhancer-binding protein signaling, or direct regulation by nuclear factor-κB or posttranscriptional mechanisms via microRNAs.[7] The principal mechanisms are through the transcription factors pregnane X receptor (PXR) and constitutive androstane receptor (CAR), leading to variation in the sensitivity of different CYPs to inflammation.[7] Indeed, CYP3A, CYP2C9, and CYP2C19

are regulated by PXR and CAR and are more sensitive to inflammation, whereas the aryl hydrocarbon receptor regulated CYP1A2 isoform is less sensitive.[7] CYP2D6 is the least affected CYP because it is not inducible by nuclear receptors and seems therefore not affected by inflammation-induced alterations.[7]

Personalized medicine aims to enable the design of a virtual representation of the patient and the development of predictive models based on known interactions between molecular, environmental, and lifestyle data by a computational algorithm as a decision support to individualize treatment.[2]

Dynamic physiologically based PK (PBPK) models are used to predict plasma concentration curves by simulating the concentration-time profiles of a drug and its metabolite(s) in plasma or in an organ of interest and simultaneously allow estimation of PK parameters.[14] The PBPK approach has been included in regulatory guidance and the development of new drugs and new drug applications.[14] The understanding of some of the causes of absorption, distribution, metabolism, and excretion (ADME) changes that occur in different disease states has improved, and consequently PBPK modeling has been used to simulate drug disposition in special populations.[14] In fact, PBPK models combined with in vitro–in vivo extrapolation allow the description of many phenomena involved in complex PK processes, integrating prior knowledge of the anatomical, physiological, and biochemical characteristics of the body as well as the physicochemical properties of the drug.[15] Therefore, the lack of in vivo data in patient populations contribute to limiting the application of PBPK modeling to predict PK in disease populations.[14]

The aim of our study was to develop a PBPK model that simultaneously characterizes the impact of IL-6 and CYP2C19 inhibition by esomeprazole on the PK of midazolam and omeprazole, two probe substrates used to assess the activities of CYP3A (CYP3A4 and CYP3A5) and CYP2C19 in patients undergoing elective hip surgery.[16] We aimed to quantitatively predict the clinical drug-drug-disease interaction on CYP3A and CYP2C19 activities.

METHODS

PK data

PK data were obtained from the raw data of our recently published cohort study, which assessed the activity of the six major CYP isoforms (cocktail approach) before and after elective hip surgery.[16] A total of 30 patients received the "Geneva cocktail" before and 1 and 3 days after surgery and at discharge (5 to 6 days after surgery).[16] The composition of this oral cocktail and the sampling and analytical methods to assess the concentration of probe substrates and their metabolites have been described previously.[16] The study population did not take CYP inhibitors or inducers, with the exception of esomeprazole in the postoperative setting, as this is a routine prescription after surgery in the hospital where the cohort study was conducted.[16]

Systemic IL-6 concentrations in surgical patients

Systemic levels of IL-6 were also systematically measured before surgery, the first 3 days after surgery, and at discharge.[16] Sample preparation and analytical methods for IL-6 determination have been described elsewhere.[16] The population mean and standard deviation (SD) of systemic levels of IL-6 were calculated for each day.

PBPK models

PBPK models of midazolam (CYP3A) and omeprazole (CYP2C19) in virtual surgical patients were developed using the ADME simulator Simcyp™ Version 19 (Certara®, Simcyp Limited). The virtual population with surgery-related inflammation was characterized by incorporating the impact of systemic IL-6 (inhibitor 1) level and esomeprazole (inhibitor 2) intake on hepatic and intestinal expressions of CYP3A and CYP2C19 of the default healthy Caucasian population. General aspects of PBPK model characteristics, enzyme dynamics, and victim drug kinetics in the ADME simulator have been described previously.[17,18] As a first step, the simulator built-in library models of midazolam and omeprazole were used in the current PBPK models to characterize the plasma concentrations of these CYP3A and CYP2C19 substrates. However, the default simulated concentration-time profile of omeprazole did not match the concentration-time profile of omeprazole in a population of Caucasian healthy volunteers (data not shown).[19] Based on a previously validated model, volume of distribution at steady state (Vss) of omeprazole was changed from 0.15 to 0.23 L/kg because the default value did not fit the Caucasian population.[20,21] The models used for midazolam and omeprazole are summarized in Table S1 and Table 1, respectively.

Modeling of midazolam metabolite

The metabolism of midazolam is almost exclusively performed by CYP3A to 1-hydroxymidazolam (1-OH-MDZ). Indeed, 1-OH-MDZ, 4-OH-MDZ, and 1,4-OH-MDZ

TABLE 1 Parameters for omeprazole and 5-OH-OMPZ

Parameters	Omeprazole	5-OH-OMPZ[25]
Molecular weight (g/mol)	345.4	361.4
logP	2.33	1.1
Compound type	Ampholyte	Ampholyte
pKa	9.33; 4.31	9.29; 3.93
B/P	0.59	0.59 (assumed)
fu,p	0.053	0.17
Absorption		
Model	First order	NA
Fraction absorbed	Predicted	NA
fu,gut	0.053	NA
Extrapolated Peff, man (10^{-4} cm/s)	12	NA
MDCK II (10^{-6} cm/s)	59	NA
Distribution		
Model	Minimal PBPK	Minimal PBPK
Vss (L/kg)	0.23[21]	0.1 (adjusted parameter)
Elimination		
Enzyme kinetics		
Cl_{int} CYP2C19 in recombinant (µl/min/pmol of isoform)	62.593	NA
fumic CYP2C19	1	NA
Cl_{int} CYP3A4 in recombinant (µl/min/pmol of isoform)	0.201	NA
fumic CYP3A4	1	NA
Cl_R (L/h)	0	0
Additional systemic clearance (L/h)	NA	45 (adjusted parameter)
$Kapp_{CYP2C19}$ (µM)	0.65	NA
$kinact_{CYP2C19}$ (h^{-1})	2.9	NA

Abbreviations: B/P, blood-to-plasma partition ratio; Cl_{int}, in vitro intrinsic clearance; Cl_R, renal clearance; CYP, cytochrome P450; fu,gut, unbound fraction of drug in enterocytes; fumic, fraction of unbound drug in the in vitro microsomal incubation; fu,p, fraction unbound in plasma; Kapp, concentration of mechanism-based inhibitor associated with half maximal inactivation rate; kinact, inactivation rate of the enzyme; MDCK II, Madin-Darby canine kidney permeability cell line; NA, not applicable; PBPK, physiologically based pharmacokinetic; Peff,man, human jejunum effective permeability; pKa, acid dissociation constant at logarithmic scale; Vss, volume of distribution at steady state; 5-OH-OMPZ, 5-hydroxy-omeprazole.

account for 75%, 3%, and <1% of the metabolites, respectively.[22] In this PBPK model, a previously described and validated model for 1-OH-MDZ was used (Table S1).[23]

Modeling of omeprazole metabolite

Omeprazole is almost exclusively metabolized by CYP2C19 in 5-hydroxyomeprazole (5-OH-OMPZ), as 60.5%, 25%, and 14.5% of the racemate is metabolized to 5-OH-OMPZ, 5-O-desmethyl-OMPZ, and OMPZ-sulfone, respectively.[24] We used a previously published and validated model as the basis for implementing this metabolite in our PBPK model.[25] However, the renal clearance (Cl_R) was changed from 0.037 to 0 L/h to be consistent with that of the built-in library model of omeprazole (Table 1).

Modeling of esomeprazole

A previously developed and validated PBPK model was used for esomeprazole, the S-isomer of omeprazole (Table 2).[25] This published model considered that

TABLE 2 Parameters for esomeprazole

Parameters	Esomeprazole as published [25]	Esomeprazole as used
Molecular weight (g/mol)	345.4	345.4
logP	2.23	2.23
Compound type	Ampholyte	Ampholyte
pKa	4.4; 8.7	4.4; 8.7
B/P	0.59	0.59
fu,p	0.03	0.03
Absorption		
Model	First order	First order
fa	1	1
ka (/h)	2	2
Lag time (h)	0	0
MDCK II Perm (10^{-6} cm/s)	59	59
Extrapolated Peff, man (10^{-4} cm/s)	12	12
Distribution		
Model	Minimal PBPK	Minimal PBPK
Vss (L/kg)	0.2	0.2
Elimination		
Cl_R (L/h)	0.037	0
Enzymes kinetics		
$Cl_{intCYP2C19}$ (µl/min/pmol of isoform)	24.3	24.3
$Ki_{CYP2C19}$ (µM)	8.4	8.4
$Kapp_{CYP2C19}$ (µM)	0.2706	0.2706
$kinact_{CYP2C19}$ (/h)	1.74	1.74
$fumic_{CYP2C19}$	1	1
$Cl_{intCYP3A4}$ (µl/min/pmol of isoform)	0.36	0.36
Ki_{CYP3A4} (µM)	40	40
$Kapp_{CYP3A4}$ (µM)	1.716	NA
$kinact_{CYP3A4}$ (/h)	1.74	NA
$fumic_{CYP3A4}$	1	1

Abbreviations: B/P, blood-to-plasma partition ratio; Cl_{int}, in vitro intrinsic clearance; Cl_R, renal clearance; CYP, cytochrome P450; fa, fraction absorbed; fu,gut, unbound fraction of drug in enterocytes; fumic, fraction of unbound drug in the in vitro microsomal incubation; fu,p, fraction unbound in plasma; ka, first-order absorption rate constant; Kapp, concentration of mechanism-based inhibitor associated with half maximal inactivation rate; Ki, concentration of inhibitor that supports half maximal inhibition; kinact, inactivation rate of the enzyme; MDCK II Perm, Madin-Darby canine kidney permeability cell line; NA, not applicable; Peff,man, human jejunum effective permeability; pKa, acid dissociation constant at logarithmic scale; Vss, volume of distribution at steady state.

esomeprazole was both a reversible and irreversible inhibitor of CYP2C19 and CYP3A4, even though its effect on CYP3A4 is not usually considered relevant in clinical practice.[8,26,27] Published data indeed suggest a short-term effect of esomeprazole on midazolam concentration but no irreversible CYP3A inhibition, even with twice the dose used for the current simulation.[28,29] The irreversible inhibition of CYP3A4 by esomeprazole (mechanism-based inhibition) was thus removed from the simulation. However, both inhibitions of CYP2C19 were kept in the model because it is accepted in the literature that esomeprazole is a CYP2C19 reversible and irreversible inhibitor.[28,29] Moreover, the Cl_R was changed from 0.037 to 0 L/h, as well as for 5-OH-omeprazole, to be consistent with Simcyp™ built-in library model of omeprazole (Table 2). Indeed, the published PBPK model of esomeprazole considered that Cl_R was similar for both enantiomers and omeprazole is a racemic mixture. The esomeprazole model was introduced as a drug inhibitor in the current PBPK models.

Modeling of IL-6 profiles

In the present PBPK model, the IL-6 model used has been previously developed and validated.[30] A number of stimulations were performed to obtain different steady-state plasma IL-6 concentrations, and we found the one that matched the mean plasma IL-6 concentrations described in the cohort study at each day. The chosen mode of administration was an intravenous infusion of 30 doses of 9×10^{-5} μg/h with a 1-h interval. As previously described, the IL-6 compound built was linked to an effect on hepatic CYP3A and CYP2C19 levels, and new steady-state levels of these CYPs were achieved during the simulation period, and the suppressive effect of IL-6 on intestinal CYP was assumed to be the same as that on hepatic CYP.[31] The final parameters used to build the IL-6 compound for our PBPK model are shown in Table S2. Information on CYP3A4/5 and CYP2C19 inhibition by IL-6 was obtained by the reassessment of data contained in an in vitro study and not by directly using the values given in the existing IL-6 model.[32] The IL-6 model was introduced as a drug inhibitor in the current PBPK models.

Modeling of enzyme dynamics

The impact of IL-6 on CYPs was modeled as a suppression of CYP3A4/5 and CYP2C19 in the liver in the ADME simulator. The equation was revised from the literature and described previously.[30]

Plasma versus dried blood spot correlation

The ADME simulator gives concentrations of substances in plasma, whereas the concentrations obtained from the cohort study are in whole blood (dried blood spot [DBS]). Therefore, a correction factor had to be applied to covert the concentration obtained in DBS into plasmatic concentration. Raw data from a published study that assessed the correlation of the concentrations of probe drugs contained in the "Geneva cocktail" between plasma and DBS were used.[33] The following equations were used:

1. $[\mathrm{MDZ_{plasma}}] = [\mathrm{MDZ_{DBS}}] \times 1.581 - 0.031$
2. $[1-\mathrm{OH-MDZ_{plasma}}] = [1-\mathrm{OH-MDZ_{DBS}}] \times 1.790 - 0.048$
3. $[\mathrm{OMPZ_{plasma}}] = [\mathrm{OMPZ_{DBS}}] \times 1.413 + 1.126$
4. $[5-\mathrm{OH-OMPZ_{plasma}}] = [5-\mathrm{OH-OMPZ_{DBS}}] \times 1.562 - 0.315$

Development and validation of PBPK model to simulate the interaction between inflammation (IL-6) and CYP3A and CYP2C19 substrates

The current PBPK models (midazolam/1-OH-midazolam and omeprazole/5-OH-omeprazole) were developed with a stepwise strategy. First, plasma concentration–time profiles of midazolam and omeprazole and their respective main metabolites were simulated in a healthy Caucasian virtual population provided by the ADME simulator, with IL-6 as the inhibitor 1. A visual prediction check was performed to evaluate the accuracy of the PBPK model prediction. Then, omeprazole Vss was changed to better match the Vss found in the Caucasian population, and the esomeprazole model was integrated as the inhibitor 2 to optimize the simulation.

The model validation process was performed by comparing the model prediction of omeprazole and its main metabolite and esomeprazole with published studies conducted in healthy volunteers. Comparison of observed and simulated concentration-time profiles of omeprazole and its main metabolite, 5-OH-omeprazole, was performed using clinical data obtained from a study conducted in our laboratory by Bosilkovska et al. (raw data not shown).[19] The esomeprazole model also needed to be validated as a previously validated model was modified.[25] We extracted the concentration-time profile of esomeprazole from one study with the same dose used in the cohort study to obtain observational data.[34]

PK parameters were estimated by standard noncompartmental methods using WinNonlin Version 6.2.1 (Pharsight) and by Simcyp™.

Once these three substances were validated, the PBPK models prediction values were compared with the observed values in the cohort study between midazolam and omeprazole and their metabolites to assess its predictability and application.

All simulations were conducted using 10 trials containing 10 subjects for 8 days. Midazolam 1 mg and omeprazole 10 mg were administrated orally at 7 a.m. on Days 1, 2, 4, 6, and 7 (custom dosage) and esomeprazole 40 mg was administrated orally at 7 p.m. on Days 1, 2, and 3. A total of 30 doses of 9×10^{-5} μg with $\tau = 1$ h of IL-6 were administrated at 9 a.m. on Day 1. Simulated plasma concentrations of midazolam and omeprazole and their metabolites at 2 h, 26 h, 74 h, 122 h, and 146 h (to account for the 2 h required after the intake of midazolam and omeprazole) were compared with concentrations obtained at baseline, 24 h, and 72 h after surgery and at discharge (5 or 6 days after surgery).

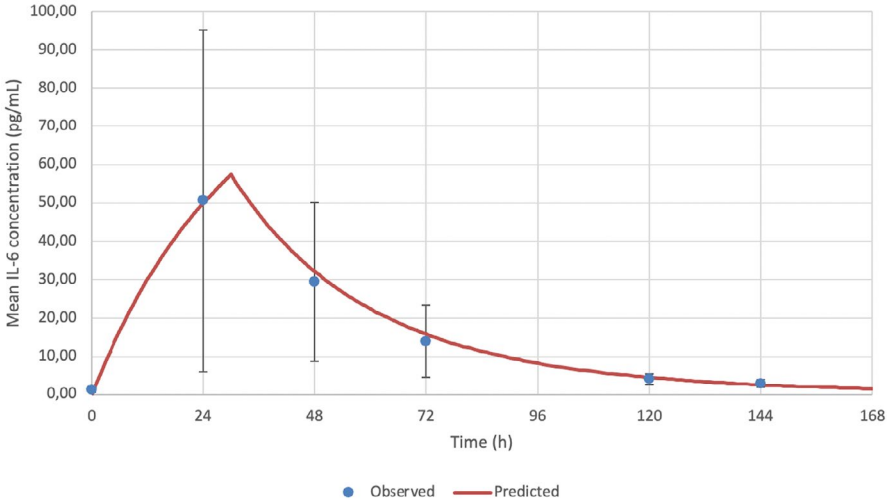

FIGURE 1 Observed concentration-time profile of IL-6 (dots) and simulated concentration-time profile of IL-6 (line). IL-6, interleukin-6

TABLE 3 Observed versus predicted pharmacokinetic parameters

	Observation	Simulation
IL-6		
Geometric mean AUC (mg.h/L)	0.0019 ± 0.0017	0.0018 ± 0.0007 (90% CI, 0.0017–0.0019)
Mean $t_{1/2}$ (h)	36.6 ± 14.7	32.6
Mean C_{max} (mg/L)	0.0001 ± 0.00004	0.0001 ± 0.00001
Omeprazole		
Geometric mean AUC (mg.h/L)	0.16 ± 0.14	0.22 ± 0.67 (90% CI, 0.186–0.267)
Mean $t_{1/2}$ (h)	1.03 ± 0.65	1.00 ± 1.15
Mean C_{max} (mg/L)	0.11 ± 0.05	0.15 ± 0.12
5-OH-omeprazole		
Geometric mean AUC (mg.h/L)	0.11 ± 0.026	0.22 ± 0.09 (90% CI, 0.211–0.239)
Mean $t_{1/2}$ (h)	1.25 ± 0.56	1.00 ± 1.14
Mean C_{max} (mg/L)	0.05 ± 0.02	0.14 ± 0.07
Esomeprazole		
Geometric mean AUC (mg.h/L)	3.87 (95% CI, 2.96–5.07)	4.11 (95% CI, 3.59–4.72)
Geometric mean $t_{1/2}$ (h)	1.25 (95% CI, 1.09–1.44)	1.35 (95% CI, 0.97–1.09)
Geometric mean C_{max} (mg/L)	1.60 (95% CI, 1.31–1.96)	1.14 (95% CI, 1.04–1.24)

Abbreviations: AUC, area under the curve; CI, confidence interval; C_{max}, maximum concentration; IL-6, interleukin-6; $t_{1/2}$, 5-OH, half-life 5-hydroxy-omeprazole.

RESULTS

Model validation of IL-6 via prediction of clinical observation

The observed and simulated concentration-time profiles were compared, and the accuracy of the PBPK model prediction was confirmed (Figure 1). The simulated and observed PK parameters are presented in Table 3. The mean area under the curve (AUC) ratio between observation and simulation was 1.05, meaning that observation and prediction are similar.

Validation of the omeprazole and 5-OH-omeprazole models

The simulated and observed PK parameters are presented in Table 3, leading to a simulated geometric mean $AUC_{0-8\,h}$ ratio of 5-OH-omeprazole/omeprazole of 1.009. The observed geometric mean AUC ratio of 5-OH-omeprazole/omeprazole was 0.66.[19] Therefore, the AUC ratio between observation and simulation was 1.53, which is the accepted range of equivalence in PBPK modeling. PBPK models of omeprazole (Figure 2a) and 5-OH-omeprazole (Figure 2b) accurately predict the observed data. The slight

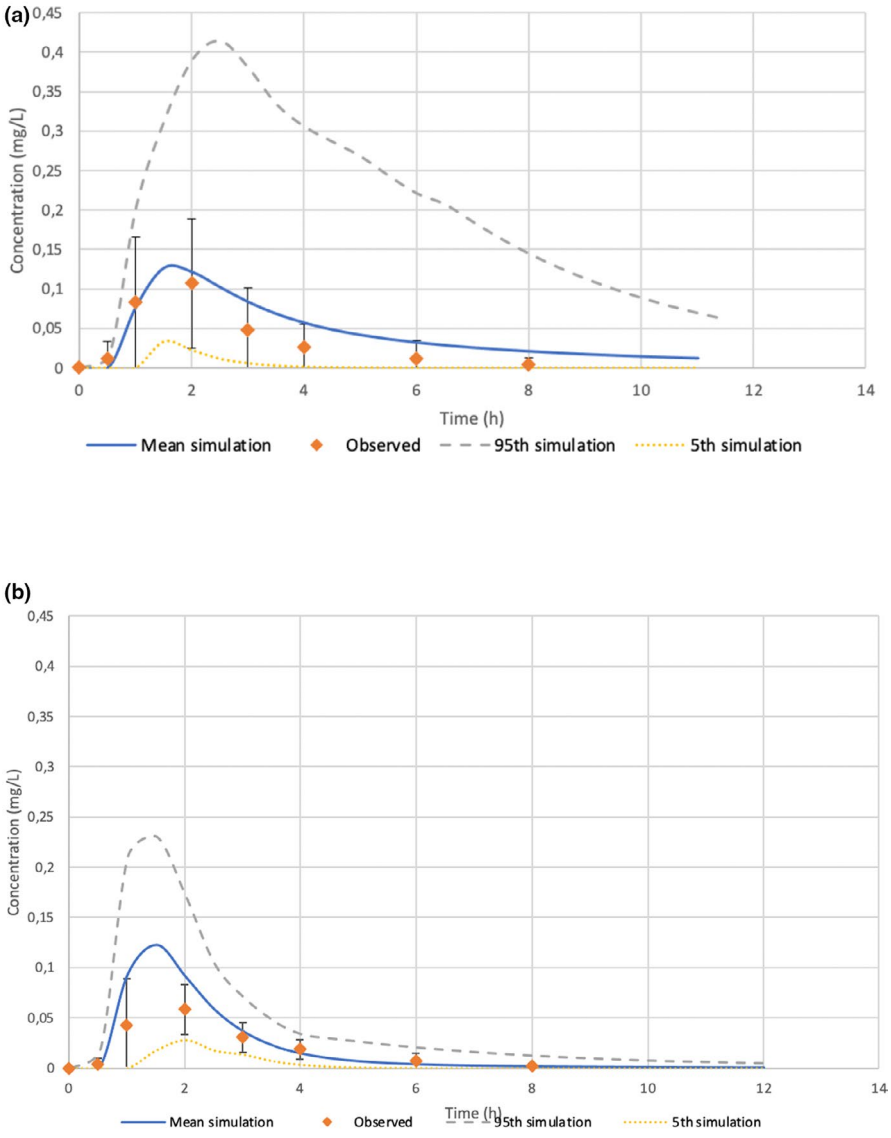

FIGURE 2 Observed concentration time-profile (dots) and simulated concentration-time profile (line) of (a) omeprazole and (b) 5-hydroxy-omeprazole

but acceptable overestimation of the simulated mean 5-OH-omeprazole concentrations could be explained by the sampling times of the training set where the point of the maximal concentration could have been missed. This was confirmed by the difference in maximum concentration (Table 3).

Validation of the esomeprazole model

Figure 3 shows the accurate prediction of the observed concentration-time profile of esomeprazole by the PBPK model.[34] The simulated and observed PK parameters are presented in Table 3. The geometric mean AUC ratio between observation and simulation was 1.06, which is in the accepted range of bioequivalence (between 0.85 and 1.25).

Verification of the performance of the PBPK models

The established PBPK models of midazolam/1-OH-midazolam and omeprazole/5-OH-omeprazole as well as those of esomeprazole and IL-6 were used to predict the effects of the mean IL-6 and esomeprazole concentration-time profiles on CYP3A and CYP2C19 activities in elective hip surgery patients. The changes in hepatic intrinsic clearance as a function of time of midazolam/1-OH-midazolam and omeprazole/5-OH-omeprazole, respectively, were thus simulated with the models. As three-quarters of the patients in the cohort study were on esomeprazole in the postoperative setting, an esomeprazole model was implemented in our PBPK models. Almost all patients took esomeprazole in the evening, so there was no interference with measuring the concentration of omeprazole

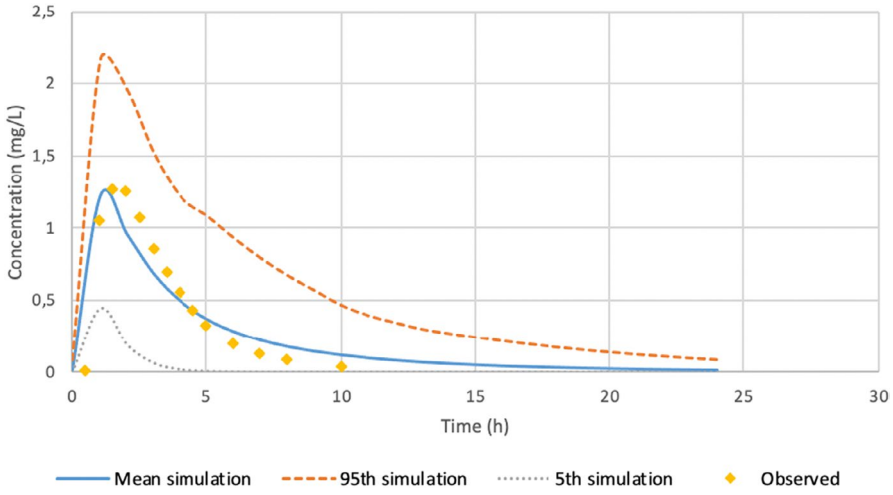

FIGURE 3 Observed (dots) and simulated (lines) concentration time-profiles of esomeprazole after 5 days of treatment with 40-mg esomeprazole

or 5-OH-OMPZ given in the morning to assess CYP2C19 activity. Indeed, esomeprazole is the S-isomer of omeprazole and the liquid chromatography with tandem mass spectrometry method used is unable to differentiate enantiomers, but its half-life is short, approximatively 1.3 h.[26] Moreover, about 27% of esomeprazole is metabolized in 5-OH-OMPZ, but its half-life is between 0.9 and 1.7 h, depending on CYP2C19 phenotype.[24,35]

Plasma concentrations of midazolam/1-OH-midazolam and omeprazole/5-OH-omeprazole at 2 h were simulated as a function of time-dependent changes in IL-6 and of esomeprazole intake. These simulated concentrations were comparable with those observed in the cohort study of elective hip surgery patients.[16] Indeed, as shown in Figure 4, the changes in the predicted mean concentrations as a function of time for midazolam (Figure 4a), 1-OH-midazolam (Figure 4b), omeprazole (Figure 4c), and 5-OH-omeprazole (Figure 4d) are within the accepted ratio of 2 to the mean observed concentration for 100% of simulated concentrations. Moreover, for 60%, 17%, 33%, and 33% of predicted mean concentrations for midazolam, 1-OH-midazolam, omeprazole, and 5-OH-omeprazole, respectively, the fold changes were less than 1.25, which is the limit of bioequivalence. The metabolic ratio (MR) versus time for midazolam (Figure 4e) and omeprazole (Figure 4f) were also within the accepted range. In addition, as shown in Figure S1, the observed mean concentrations 2 h after "Geneva cocktail" intake versus time were close to the simulated mean concentration versus time profile. The comparison between observation and prediction shown in Figure S1 can only be made with one observed time-point because it is a concentration obtained after phenotyping (MR 2 h after administration of the "Geneva cocktail"). Figure S2 shows that the time-varying IL-6 concentrations and esomeprazole intake decrease CYP3A and CYP2C19 activities. Moreover, CYP2C19 activity without both inhibitors was not 100% during the first days of the study because omeprazole was administrated periodically until Day 7 to assess CYP2C19 activity and it inhibits its own metabolism. CYP2C19 and CYP3A activity returned to 100% when no further CYP2C19 and CYP3A inhibitors were administered to patients. Indeed, esomeprazole and the probe drugs (midazolam and omeprazole) were no longer administered after Day 3 and Day 7, respectively, and IL-6 levels gradually decreased (Figure 1). A return to baseline could therefore be expected after approximatively 12 days (Figure S2).

Comparing simulated drugs concentrations with and without drug-drug interactions (esomeprazole) and drug–disease interactions (IL-6) in the investigated clinical studies obtained at every hour throughout the study, there were 2.1 ± 0.3-fold, 1.6 ± 0.2-fold, 3.1 ± 0.6-fold, and 3.2 ± 0.6-fold (mean ± SD) increases in simulated concentrations of midazolam, 1-OH-midazolam, omeprazole, and 5-OH-omeprazole, respectively.

DISCUSSION

A virtual surgery population was developed and validated to assess the impact of surgery as a source of variability in drug effects and to predict the changes in the PK profiles of concomitant treatments in the postoperative setting. We used observed data from a real-life study conducted in our center in elective hip surgery, where CYP activities were evaluated using a cocktail approach to build the model.[16]

The prediction of IL-6-mediated drug–disease interaction via PBPK modeling had been previously described in the literature.[30,31,36–39] Indeed, PBPK models were developed to predict the impact of elevated levels of IL-6 on CYP substrates using a cocktail approach

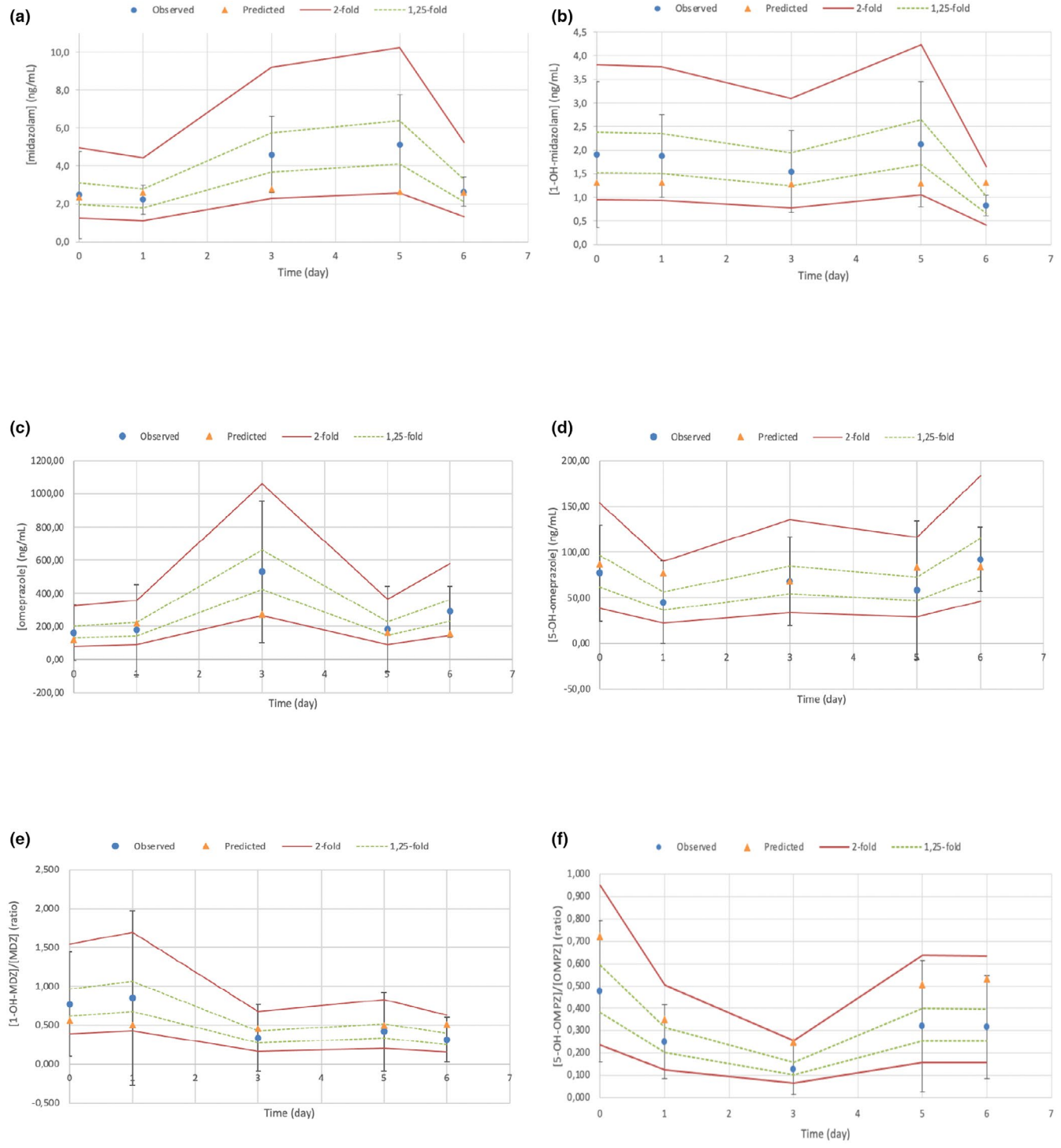

FIGURE 4 Concentration versus time profiles (a-d) and metabolic ratio vs time profiles (e-f) for observed and predicted values and corresponding fold changes of 2 (lines) and 1.25 (dashed lines), 2 h after "Geneva cocktail" intake in the presence of time-varying interleukin-6 concentrations and esomeprazole intake for (a) midazolam, (b) 1-hydroxy-midazolam, (c) omeprazole] (d) 5-hydroxy-omeprazole, (e) 1-hydroxy-midazolam/midazolam, and (f) 5-hydroxy-omeprazole/omeprazole

with probe drugs or assessing simvastatin, rivaroxaban, and vancomycin PK in different special populations (rheumatoid arthritis, neuromyelitis optica, or critically ill sepsis).[30,31,36–39] They used in vitro data to quantitatively predict the intensity of the clinical drug–disease interaction via IL-6, which appears to be the key element in modulating CYP activities during inflammation.[7,32,37,40,41]

In our study, PBPK models were developed for omeprazole and midazolam and their main metabolites using

whole-blood (DBS) data and drug-specific correction factors to convert DBS to plasma concentrations.[33] Indeed, Simcyp™ uses the plasma concentration. To our knowledge, this is the first time that concentrations from whole blood have been used in Simcyp™.

We confirmed that omeprazole by default (from the Simcyp™ built-in library) does not match the Caucasian population.[19] According to the literature, the Vss of omeprazole varies with the ethnicity of the population.[21] Our observed data population study was Caucasian, so the Vss value was changed accordingly from 0.15 L/kg to 0.23 L/kg.[20,21] Our new model was consistent with the values observed in the literature.[19]

As our cohort study did not give complete PK of midazolam and omeprazole, it was important to build a model for the main metabolites of midazolam and omeprazole to increase the confidence and predictability of the developed models.

Midazolam is mainly metabolized by CYP3A to 1-OH-midazolam.[22] We used a previously published and validated model of 1-OH-midazolam.[23]

Omeprazole is mainly metabolized in 5-OH-omeprazole by CYP2C19, and we thus designed a model for 5-OH-omeprazole into the current PBPK model.[24] We adapted an existing model by changing the Cl_R as it was assumed that 5-OH-omeprazole Cl_R was the same as that of omeprazole.[25] This new 5-OH-omeprazole plasma concentration is consistent with data observed in the literature.[19]

Esomeprazole is a well-known CYP2C19 inhibitor that was systematically prescribed in our cohort study to prevent the occurrence of stress ulcer, although we would have liked to exclude all CYP modulators.[26] To simulate the impact of inflammation attributed to surgery on CYP2C19 activity, we thus considered CYP2C19 inhibition by esomeprazole.

As with omeprazole, we changed the Cl_R to 0 L/kg. The published model for esomeprazole used both a mechanism-based and a reversible CYP3A inhibition by esomeprazole.[25] We removed the mechanism-based inhibition of CYP3A by esomeprazole, leaving only reversible inhibition as the literature does not report irreversible inhibition of CYP3A by esomeprazole.[28,29] Based on the validation results, our esomeprazole model was consistent with the data observed in the literature.

The disease–drug interaction is complex and depends on a multitude of factors that are not always known, making in vitro–in vivo extrapolation difficult. Indeed, studies have reported that the onset of inflammation impacts the levels of other proteins than cytokines and that they influence the PK parameters of drugs.[10,42,43] Also, some evidence suggest that cytokines may modulate the transporter activities that also play a role in the ADME process.[10,42] Moreover, DDIs have multiple sources, as multiple PK and pharmacodynamic (PD) factors can interfere.[25] PBPK modeling can help evaluate DDIs without clinical trials and is used in drug development.[44,45]

Our model has allowed the innovative integration of a PD biomarker, IL6, as a marker of inflammatory response. The development and use of PBPK models go beyond the prediction of PK properties of a drug as they have been used to predict and assess drug efficacy and safety.[46] Other perspectives are to combine PK models with the corresponding PD response as we have successfully done in our model.[46,47] Our PBPK approach successfully predicted the modulation of CYP3A and CYP2C19 activities by IL-6 (drug–disease interaction) and esomeprazole (DDI). Improvements will, however, have to be made to bring all predicted values within this bioequivalence range to introduce it into clinical practice. These simulations showed the importance of this type of approach to support personalized medicine, as the interactions increased midazolam and omeprazole concentrations by twofold and threefold, respectively, which may lead to efficacy and safety concerns. Because of the increase in life expectancy, patients are medically more complex due to a greater number of comorbidities and, consequently, comedications.[48] Model-informed precision dosing (MIPD) allows for the prediction and selection of the correct dose considering the contribution of covariates to reduce the variability of a target concentration as clinicians must deal with variability in many ways.[48] The development of MIPD could fulfill the need in clinical practice to facilitate interpretation and decision making toward the abundance and complexity of data in clinical care, such as complex drug-drug-gene-disease interactions influencing drug efficacy and safety.[48]

Smart, easy-to-use, and clinically validated MIPD tools could integrate all of these factors and enable optimal drug use, leading to the improvement of health outcomes, decrease of drug-related harm, and smaller economic burden.[48] To the best of our knowledge, our PBPK models are the first models to include both DDI and the drug–disease interaction, and this is a step forward in the development and use of MIPD to achieve precision medicine. In addition, these PBPK models may be useful to complement those developed in emerging therapeutic areas, such as chimeric antigen receptor T (CAR-T) and T cell–redirecting bispecific antibody therapy, as patients have also reported temporary elevation of cytokines, including IL-6, following treatments.[37,49] This approach has the potential to be extended to provide dosing guidance for concomitant medications during such treatments.

Our study has some limitations. First, the data used to inform the PBPK model of in vitro IL-6 suppression of CYP3A and CYP2C19 came from a single study. Moreover, our model only considered the suppressive effect of IL-6

and not the impact of other cytokines or acute-phase proteins such as C reactive protein. However, as mentioned previously, IL-6 is considered to be the critical cytokine responsible for the downregulation of CYP activities. This suggests that incorporation of other inflammatory biomarkers would result in only minor changes. Another important limitation is that we used clinical data from a study that was not designed to provide complete concentration-time profiles, but only the concentrations of probe substrates 2 h after oral administration. It would have been more accurate and meaningful to compare concentration-time profiles between simulated and observed data. In an attempt to compensate for the lack of comparators, we also simulated the major metabolites of midazolam and omeprazole produced by CYP3A and CYP2C19, respectively. Another limitation inherent in the cohort study was that the follow-up period was not long enough to see a return to baseline levels of CYPs. Indeed, the majority of included patients were discharged from the hospital 3 days after surgery and never more than 6 days after.[26] Finally, it was not possible to discriminate between the inhibitory effect of IL-6 or esomeprazole on CYP2C19 because it was a routine postoperative treatment.[26]

CONCLUSION

Inflammation is a transient or chronic health condition, inducing transient or chronic physiopathological changes, which may impact on drug PK parameters. PBPK simulations allow varying system parameters and incorporating literature-based alterations in CYP activities due to inflammation. The current model successfully predicted midazolam and omeprazole and the PK of their main metabolites in a population with surgery-related acute reversible inflammation. Moreover, the integration of the esomeprazole model resulted in a better fit for omeprazole PK. This study could be a basis for refining dosing recommendations in the postoperative setting, especially in drugs with narrow therapeutic indexes. In fact, PBPK models may be an effective and efficient way to investigate the risk of interaction using existing knowledge about the distinctive characteristics of the disease population. The integration and prediction of PD and disease parameters in PBPK models thus appears to be a promising approach to personalize treatments.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

C.L., Y.D., and C.F.S. wrote the manuscript. C.L., A.N., V.R., J.A.D., Y.D., and C.F.S. designed the research. C.L. and A.N. performed the research. C.L., A.N., Y.D., and C.F.S. analyzed the data. C.L., A.N., and Y.D. contributed new reagents/analytical tools.

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SUPPORTING INFORMATION

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Table S1: Parameters for midazolam and 1-hydroxy-midazolam.

Parameters	**Midazolam** (Simcyp™ built-in library)	**1-OH-MDZ** [23]
Molecular weight (g/mol)	325.8	342
logP	3.53	2.5
Compound type	Base	Ampholyte
pKa	6.0	13.6; 3.63
B/P	0.603	1 (assumed)
fu,p	0.032	0.15
Absorption		
Model	1st order	NA
Fraction absorbed	1	NA
ka (h^{-1})	3	NA
fu,gut	1	NA
Caco-2 permeability (10^{-6} cm/s)	213 (7.4 apical and basolateral pH)	NA
Extrapolated Peff,man (10^{-4} cm/s	12	NA
Distribution		
Model	Minimal PBPK	Minimal PBPK
Vss (L/kg)	0.88	1.8
Elimination		
Cl_R (L/h)	0.085	0
Enzyme kinetics		
1-OH via CYP3A4 (recombinant)		
Vmax (pmol/min/mg of isoform)	5.23	NA
Km (µM)	2.16	NA
fumic	1	NA
4-OH via CYP3A4 (recombinant)		
Vmax (pmol/min/mg of isoform)	5.2	NA
Km (µM)	31.8	NA

fumic	1	NA
1-OH via CYP3A5 (recombinant)		
Vmax (pmol/min/mg of isoform)	19,7	NA
Km (µM)	4.16	NA
fumic	1	NA
4-OH via CYP3A5 (recombinant)		
Vmax (pmol/min/pmol of isoform)	4.03	NA
Km (µM)	38.4	NA
fumic	1	NA
1,4-diOH-MDZ (pathway 2)		
$Cl_{int,\ CYP3A4}$ *in HLM (µL/min/mg protein)*	NA	7.9
fumic	NA	0.69
1'-OH-MDZ Glucuronide (pathway 1)		
$Cl_{int,\ UGT}$ *in HLM (µL/min/mg protein)*	NA	2.9
fumic	NA	0.015

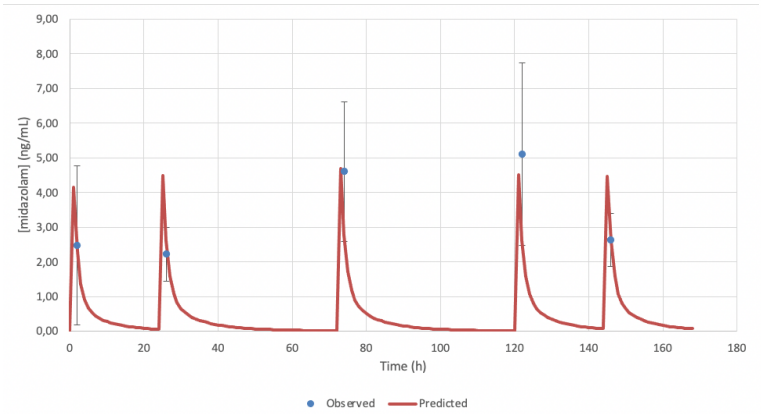
NA = Not applicable; B/P = blood-to-plasma partition ratio; fu,p = fraction unbound in plasma; ka = first-order absorption rate constant; fu,gut = unbound fraction of drug in enterocytes; Peff,man = Human jejunum effective permeability; Vss = volume of distribution at steady state; Vmax = Maximum rate of metabolism; Km = Michaelis-Menten constant; fumic = fraction of unbound drug in the in vitro microsomal incubation; Cl_{int} = in vitro intrinsic clearance; Cl_R = renal clearance

Table S2: Parameters for IL-6.

Parameters	**IL-6** [30]
Molecular weight (g/mol)	21'000
logP	0.01
Compound type	Neutral
B/P	1
fu,p	1
Absorption	
NA	
Distribution	
Model	Minimal PBPK model
Vss (L/kg)	0.43
Elimination	
Cl_{iv} (L/h)	1.00
Cl_R (L/h)	0
Enzymes kinetics	
$Ind_{max, CYP3A4}$	0.2406
$IndC50_{CYP3A4}$ (µM)	3.4841×10^{-6}
$Ind_{max, CYP3A5}$	0.0343
$IndC50_{CYP3A5}$ (µM)	2.42857×10^{-6}
$Ind_{max, CYP2C19}$	0.214
$IndC50_{CYP2C19}$ (µM)	3.39524×10^{-6}

NA = Not applicable ; B/P = blood-to-plasma partition ratio; fu,p = fraction unbound in plasma; Vss = volume of distribution at steady state; Cl_{iv} = intravenous clearance; Cl_R = renal clearance; Ind_{max} = maximal fold induction/suppression over vehicle; IndC50 = test compound concentration that supports half maximal induction/suppression

(a)



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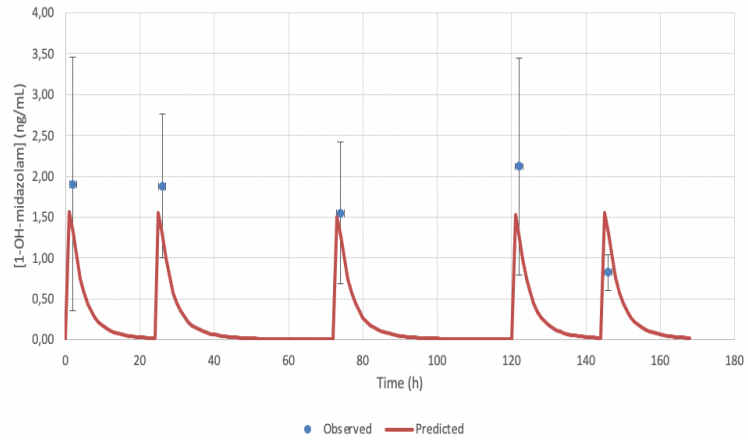

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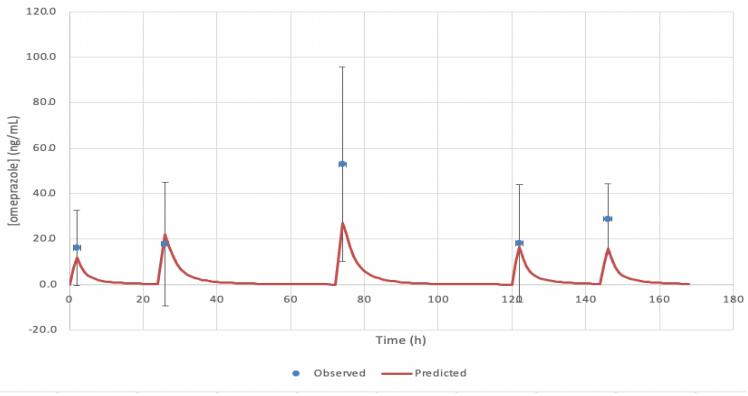


(d)

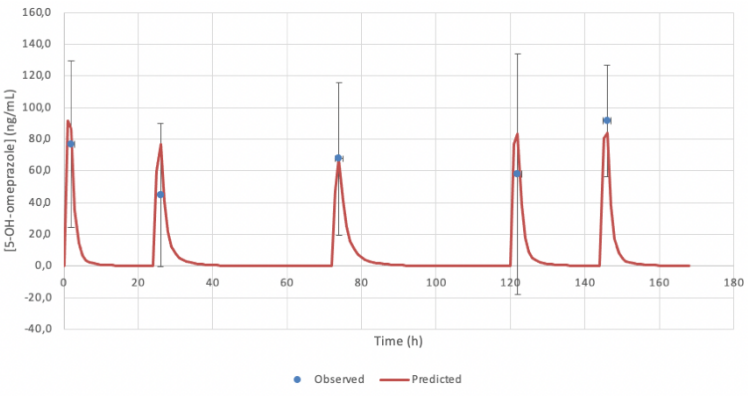
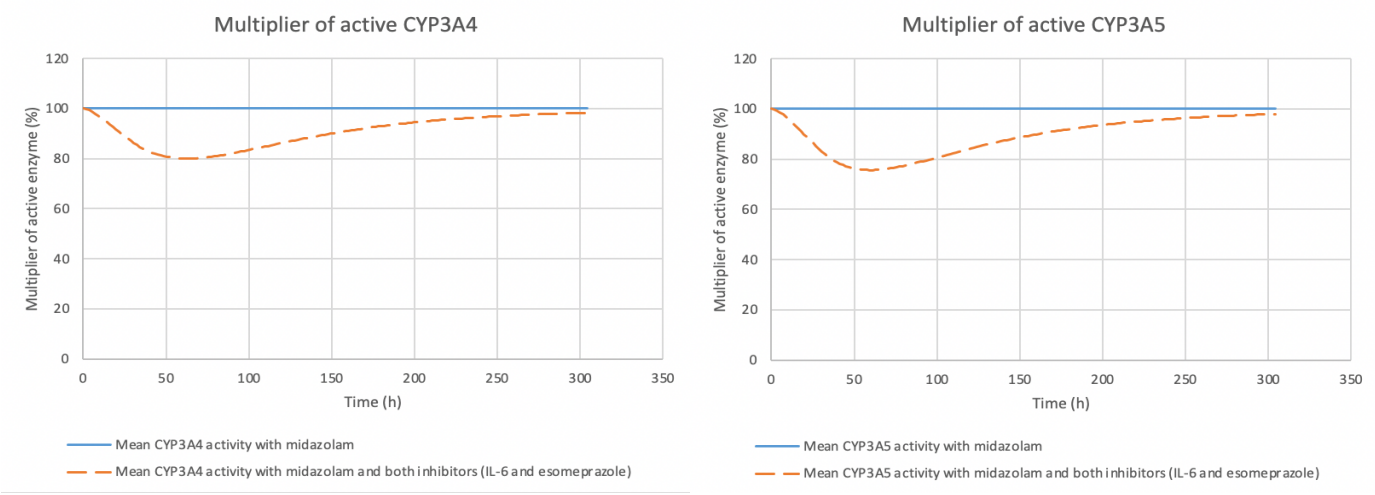


Figure S1: Concentration vs. time profiles for observed and predicted values. Observed concentrations were obtained 2h after "Geneva cocktail" intake in the presence of time-varying IL-6 concentrations and esomeprazole intake for: (a) [midazolam], (b) [1-OH-midazolam], (c) [omeprazole] and (d) [5-OH-omeprazole]

(a)



(b)

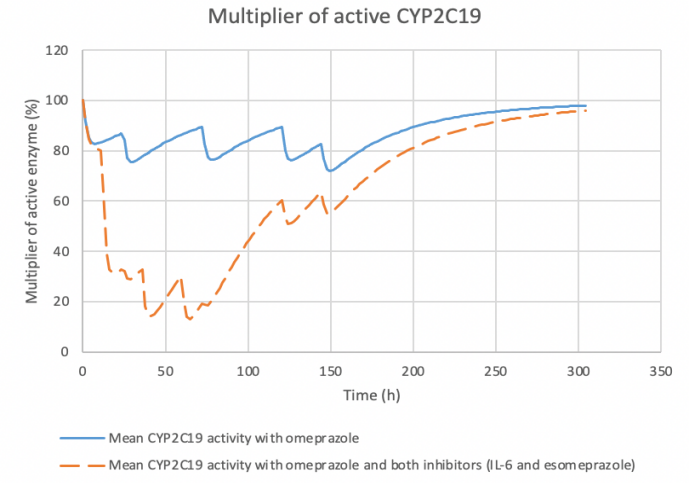


Figure S2: CYP activity profile following time-varying IL-6 concentrations and esomeprazole intake for: (a) CYP3A4 and CYP3A5 and (b) CYP2C19

Chapter 9: Discussion and Conclusion.

9.1 Discussion

Drug concentrations must be within a range below which there is no therapeutic benefit and above which there are harmful effects [1]. However, the early detection of safety and efficacy concerns has been problematic, leading to marked risks in certain populations [2]. Pharmacovigilance helps to detect earlier the occurrence of adverse events in terms of safety and efficacy. Real-world data generated through pharmacovigilance processes are used for safety signal assessment, risk management and studies to support a continuous benefit-risk evaluation [3]. Regulatory decisions across the product life cycle are more and more supported by this type of data [3]. Indeed, safety must be understood in the context of how care is provided rather than under the rigid and greatly monitored circumstances of the clinical trial [3]. In this thesis, we observed that the benefit/risk balance of a drug might be very different according to the individual patient. Safety and efficacy profiles determined through clinical trials should be thus taken cautiously.

The first limitation of clinical trials that was observed in this thesis is the gap in transparency/disclosure in the published pharmacovigilance data [4]. It leads to disparities between SmPC and published literature, as phase I and IV studies disclosure depends on the transparency policies of drug manufacturers and the consequences on drug marketing authorization, respectively [5,6]. However, the clinical pharmacology reviews provided by the FDA sometimes discloses this information.

A second limitation also detected in this thesis and that impacts results interpretation is the low clinical enrollment [7]. Globally, it is admitted that less than half of the studies achieve their recruitment targets, in terms of sample size or duration [8,9] . This has a significant scientific, ethical and financial impact on all stakeholders and society since it leads to failure to obtain the required evidence to evaluate safety, efficacy and effectiveness of healthcare interventions in a statistically significant manner [8,9].

Finally, clinical trials suffer from other limitations inherent to their structure and the stage of clinical development [10]. It is impossible to explore all potential synergistic effects or to conduct trials on a population large enough to detect rare adverse events, except during post-marketing surveillance (phase IV studies) [10,11]. Moreover, discrepancies between study participants and patients treated in routine practice often exist due to the broad inclusion and exclusion criteria for clinical trial enrollment excluding patients with varying degrees of disease (especially renal and hepatic dysfunction) and extremes of age (pediatric and elderly) [7,12]. The problem is that the safety and efficacy of a drug are assessed by an overall beneficial response in clinical trials, but it does not mean that all patients benefit from it [3]. Real-life studies and pharmacovigilance are an alternative to drug safety and efficacy observation in a

more heterogenic population [9]. For instance, this thesis highlighted that real-world data allowed to determine that rivaroxaban and apixaban are subject to a significant amount of relevant PK and PD DDIs, especially with DMET modulators.

Real-world data is a relevant opportunity and challenge to support clinical research and development [13]. Pharmacovigilance could be reorganized to move from a drug-centered to a patient/population centered approach and give the ability to adjust drug therapies within the contexts of care and the life of patients [8]. By permitting to have high-volume data that are more representative of the diversity of treated patients, real-world data allow discovering or validating new markers for patient stratification and targeted therapies [14–16]. Stratification according to specific traits, behaviors or genetic information, permits the combination of precision medicine and public health into precision public health [15]. Disease surveillance and signal detection, risk prediction, targeting treatment interventions, and study of disease are the field in which Big Data can enhance precision in public health [15]. Therefore, the development of pharmacovigilance impacts public health and therapeutic management by improving intervention and treatment outcomes in the population [15,17]. The advent of precision medicine brings new challenges to healthcare systems [16]. Indeed, healthcare is conventionally publicly funded and these funds are allocated to treatments that benefit the maximum number of people in terms of safety, efficacy and efficiency, excluding therapies that help only few patients [16,18].

However, many biases in ICSRs reporting exist, while accurate documentation of individual cases is determinant to have a clinically and scientifically relevant conclusion [17,19]. We observed in this thesis that data stored in such databases are heterogeneous, lack completeness and do not always contain a causality assessment. Despite many years of effort and the constant publication of suggestions to improve spontaneous reporting, the quality of the data still constitutes a significant obstacle to the identification of new ADRs [19].
All the limitations inherent to spontaneous reporting systems have led to evaluate more effective and efficient ways to identify and report new safety signals [20]. This is possible with the major developments in technological abilities, such as internet, big data, advanced analytics, robotics and artificial intelligence, and with the availability of data in an electronically accessible manner, stored in one or multiple databases [20]. However, the current approach is unsustainable and does not fit the purpose anymore due to the abundance and accessibility of data and needs to be rethought [20]. In fact, our society has reached a point in which our capacity to gain information from the data without computational support is exceeded by the amount of data [15]. It is not just about applying new tools to the old concepts of ICSRs [20]. It is about performing rigorous and ingoing benefit-risk assessments that are meaningful for all

actors and can be translated into useful information to support decision making about treatment [20]. The ideal future safety system would be read directly from the original source by an artificial intelligence engine with large-scale data analytics to avoid the loss of available data by normalizing them [20]. The Sentinel initiative is moving towards this goal by giving FDA rapid and secure access to electronic health care data for almost 200 million patients from multiple data partners [20]. Nevertheless, a very smart machine would be needed to continuously access, read, evaluate and analyze real-world data from electronic medical records, claims data sets, literature, registries and even social media, to finally identify patterns and trends to uncover new knowledge [20]. For instance, the association of artificial intelligence-driven methods to extract information and learn from it is more and more applied [10]. It avoids human error, reduces manual effort, standardizes processes and accelerates processing cycle times [21]. New pharmacovigilance approaches and technologies could permit an earlier detection of drug toxicity or resistance [1]. Pharmacovigilance efforts are focused on identifying, collecting, evaluating and transforming relevant data into usable and shareable safety reports [20].

Overall, accessibility of data, implementation of electronic health record systems and expansion of machine learning approaches have transformed the field of pharmacovigilance [10].

Big data analysis must be tempered to analyze, synthesize and harmonize links between disciplines into a coordinated and coherent whole [4]. Indeed, a multidisciplinary approach is mandatory to predict appropriate dose-response in a particular individual [22]. Drug safety must also move from a mostly observational to a more predictive science, leading to a better prediction of a drug's potential to cause an adverse event, better preclinical screens and clinical diagnostics, better understanding of individual patient risks and gain biological/mechanistic insight to help validate potential post-marketing safety signals [23]. Some of the proposed solution are, among others [19]:

- To make available negative or inconsistent data from research trials
- To improve quality of spontaneous reports
- To have reports evaluated by a network of experts to avoid neglecting unintended effects of drugs
- To gain a causal mechanistic understanding of particularly informative ADR
- To increase patient involvement and motivation in spontaneous reporting
- To harness the resources available about new technologies and social media
- To use pharmacogenomics as a tool for identifying shared determinants leading to ADRs

By definition, pharmacovigilance and pharmacogenomics aims are to understand the source of heterogeneity in drug PK/PD profile and, therefore, the safety or efficacy problems [2,14,24]. To achieve a systemic exposure similar to that reached at the standard dose in a normal population, potential PK alterations in a special population should be identified [8]. The integration of both concepts in one, entitled pharmacogenovigilance, could inform and guide each other [2]. It satisfies the current need to evaluate and monitor drugs according to individual characteristics to determine better tolerated individualized treatment regimens [24,25]. Therefore, the individual clinical management of the different individual patients could be helped by databases on ADRs, DDIs and genetic polymorphisms, coupled with the rapid evolution of genomic technologies [4,18,24].

Precision medicine promises to end the traditional « one size fits all » approach by prescribing genotype-based individualized therapy to make drugs safer and more effective [26]. The drug response disparities observed between an individual with a different DME genotype led to the initiation of large-scale population-based pharmacogenetic association studies to identify possible associations between genotype and clinical outcomes, especially concerning high-profile drugs [27]. These studies assume that the genotype of all included patients predicts their functional phenotype and that the broad variability in DME genotypes leads to simple binary clinical outcomes coupled to distinct genotype groups [27]. As a result, many DGIs are now listed and a change in prescription may be required for particular genotypes and drugs [28]. These genotypes are called actionable genotypes [28]. The essence of precision medicine is the application of pharmacogenetics to therapeutics, but genetic characteristics to predict either genetic susceptibility to a disease or drug response must be distinguished [26]. Indeed, genetic markers are extremely relevant in predicting the probability of monogenic diseases but their role in predicting drug response is not as accurate [26]. This could be explained by PK differences inducing disparities in clinical outcomes only for drugs with a reliable concentration-response relationship, a narrow therapeutic index, and metabolized through one single pathway [26]. Moreover, the functional consequences of most of the detected polymorphisms have not yet been assessed, which implies that genotyping prior to any information on the functional consequences of known and unknown polymorphisms would certainly be useless, as highlighted by some authors [29,30]. It may be valuable to screen study subjects for specific genetic polymorphisms before inclusion to ensure that the study sample is representative of the general population and to determine whether safety or efficacy concerns can be attributed to a group of metabolizers [29]. For instance, the sample size calculation of a study presented in this thesis was based on the distribution of a certain genotype in the population [31].

Overall, precision medicine has not yet reached its goal, even though studies on pharmacogenomics have been extensively published [32]. Some authors believe that the lack of specific guidelines on how to select drugs and dosing regimens based on genetic test results slowed down the application of pharmacogenetics in clinical practice [26]. Others also argue that better clinical evidence and the establishment of added value are needed and that, at present, genetic tests are overvalued in relation to what they can actually provide [26].
For instance, we were unable to state in our study if the non-significant impact of the genotype on drug exposure came from not testing the SNPs that had an influence, or if non genetic factors had an impact on the PK [31]. This highlights other limitations of genotyping tests. First, unknown or new SNPs cannot be tested, leading to association studies examining only prevalent alleles [27,30]. Secondly, genotype-focused association-studies may not find strong associations between a known genotype expressing a known activity and a clinical outcome because phenoconversion due to the influence of non-genetic factors is not considered [28,30]. Using the predicted phenotype from genotype to individualize treatment in clinical practice becomes inaccurate when non-genetic factors are present, such as concomitant treatments intake or comorbidities [28]. This thesis and numerous studies or reviews have identified several mechanisms of complex interactions, leading to misclassification of predicted phenotype, in addition to limitations due to unknown genotype [33].

The influence of the genotype-phenotype mismatch induced by co-medication is not always taken into account even though it represents a risk for the patient [27]. It is now fully recognized that DDIs are responsible for ADRs, which can be identified by drug interaction databases and therefore help health professionals to prevent them [28]. DDIs can mimic genetic defects or modify metabolism [27,34]. When a CYP-dependent DDI is assessed, the CYPs inhibition or induction potential of perpetrator drugs, and the substrate specificity of victim drugs must be considered in addition to genetic polymorphisms and phenoconversion caused by sources other than DDIs [28].
DDIs are usually identified in the clinical setting as the more common source of phenoconversion [27]. But pathophysiological factors may also lead to a genotype-phenotype mismatch. Therefore, it is fundamental to distinguish between association studies conducted with healthy volunteers and patients [27]. This thesis highlighted that inflammation impacts DMET activity and expression. Therefore, the inflammatory status should be taken into account when phenotype is predicted from genotype and when drugs with a narrow therapeutic index are used [35]. In addition, we observed that cytokines released are different according to the disease, partly explaining the heterogeneity observed in terms of magnitude, time-course and CYP isoform involved. Inflammation should thus be taken into consideration in future studies

as an independent covariate, and further studies are needed for some under-investigated diseases as extrapolations between illnesses are inaccurate. In addition, many mechanisms explaining the impact of cytokines on CYPs activity are known today, but the mechanisms responsible for the impact of the CRP are still discussed [35].
This thesis also highlighted that data on the impact of resolution of inflammation on DMET activity, either by treatment or spontaneous evolution, are scarce and that longer follow-up periods are needed. We also observed that the severity of the disease seems to have an impact on the magnitude of the interaction, and careful monitoring should be applied to avoid underexposure with inflammation resolution. Overall, a disease induces many physiological changes which could have an impact on PK and PD properties of a drug.

The wrong prediction of DMET phenotype from genotype might lead to safety and efficacy concerns, depending on the pharmacological activities and the therapeutic index of the parent compound and its metabolites [27,28]. Whereas phenotyping has not benefited from the same enthusiasm as genotyping, it seems to give more personalized information [27]. For instance, the Geneva cocktail was useful in clinical practice to explain inefficacy/low drug levels or ADR/high drug levels in most cases [33]. However, its preemptive use only represented 18% of cases [33]. It shows that there are still opportunities to improve the uptake of phenotype testing in the clinics. Despite the development of many useful cocktails with good tolerability and without DDIs, cocktail approaches still have some disadvantages [30,36]. Some of them are the rare occurrence of adverse events linked to the administration of an exogenous probe and the time-consuming sampling and analysis [30,37]. The administration of several probes is furthermore not always possible in vulnerable populations and the measurement of endogenous biomarkers appears to be risk-free, in addition to saving time and money [38].
Biomarkers are defined as « a measure that characterizes, in a strictly quantitative manner, a process, which is on the causal path between drug administration and effect » [38]. Pharmacometabolomic is thus an emerging research area that aims to identify endogenous metabolites/biomarkers to explain the causal relationship between drug dose and patient's outcome [39]. Its promise is to be a valid alternative to pharmacogenomics in predicting PK, as it provides a snapshot of the phenotypic status of an individual, resulting from environmental, physiological, pathophysiological and genetic factors [38]. Metabolomic approaches using endogenous compounds are hopeful methods to overcome the drawbacks of phenotyping [32,38]. Through metabolomics, endogenous compounds have been identified as phenotyping probes to assess CYP1A2, 2C19, 2D6 and 3A activities [40,41].
Pharmacometabolomics is in its infancy and research is currently undergoing to develop phenotyping tests, in a context where the phenotype of DME better determines the drug response [27,28]. This brings the questions of the added value of pharmacogenomics and

genetic variables as biomarkers of drugs safety and efficacy as compared to phenotyping [26,27].

The answer it that genotyping should not be left behind because genotyping and phenotyping are complementary approaches [27,28,33]. Indeed, studies showed that doing both tests simultaneously explain more clinical events than each of the tests done separately [28,33]. Safety and efficacy of a drug depend on multiple factors, that can be improved by genotype testing, but not only [26]. Indeed, an interplay between genotype and phenotype exists and interactions between genes and environmental factors are complex [27]. For instance, phenoconversion induces wrong prediction of phenotype from genotype on one hand, but the impact of phenoconversion depends on the genotype on the other hand [33]. The interdependency between genotype and phenotype could be explained by [33]:

- The enhancement of the magnitude of the interaction due to a genetic variant directly impacting the CYP isoform
- The increase in vulnerability to phenoconversion due to a genetic variant directly affecting the inhibited/induced metabolic pathway
- The increase exposure of the perpetrator caused by a genetic polymorphism
- The modification of the relative contribution of a minor pathway by a genetic variant affecting the major one

Some studies have shown that the impact on clinical outcome is greater when considering DDIs than DGIs, but a synergistic effect exists between both (DDGIs) [27]. Some DDIs become clinically relevant only in the presence of genetic polymorphisms, and the inverse is true as well [28]. Moreover, genetic polymorphism could impact the existence of multiple biotransformation pathways, changing their relevance for the drug studied [28]. This refers to drug-gene-gene interactions (DGGIs). This thesis showed that DDIs should be evaluated concomitantly to genotype, because DDIs are a confounding factor.

The same conclusions concerning DDGIs and DGGIs can be applied to disease-gene associations. This thesis demonstrated that inflammation has different impacts on CYPs according to their baseline activity due to genetic and non-genetics factors, known or unknown. Moreover, we highlighted that the impact of inflammation is different between adults and children, and is very heterogeneous in the latter, due to different baseline activity of CYPs. More studies should be conducted in this particular population, as it appears that environmental factors do not have the same influence according to age.

Overall, we recommend that genotyping and phenotyping are used as complementary approaches to individualize treatment through the identification of patients at risk of having ADRs or ineffective treatment [34]. The acknowledgement of DGIs, DDGIs and DGGIs in

clinical practice may help precision medicine and demonstrates the added value of pharmacogenomics [28].

Due to polymorbidity and polymedication, patients clinical situations are more and more challenging and difficult to manage due to complex drug-drug-gene-disease interactions (DDGDIs), as underlined in this thesis [46]. The complexity and profusion of clinical data have led to the development of tools to understand these data [46]. The idea that modeling and simulation can advise precision medicine is not new, dating back to the late 1960s, but it has reemerged with the field of model-informed precision dosing (MIPD) [46]. MIPD aims to achieve the optimal balance between efficacy and toxicity for the individual patient by considering available knowledge [47]. It includes information about the disease that the patient is treated for (drug-disease interactions), his comorbidities and his routine treatments (DDIs) [47]. The final goal is to improve drug treatment outcomes in patients by including various modeling approaches [47]. Current dosing guidance can easily include one covariate but it is still uncommon to encounter dosing recommendation for special populations where more than one covariate is considered [47]. MIPD could have a significant impact in different situations such as [47]:

- When clinical data in a specific population are lacking
- With narrow therapeutic index drugs
- When allometric scaling methods are inadequate (such as young children and obese patients)
- When there is an impact of disease or treatment on drug efficacy/safety
- When there are costs associated with overdosing of an expensive drug
- When proportion of the specific subpopulation in the target population and their vulnerability is unusual

PBPK models can be used in the context of MIPD as useful tools to predict the correct dosing regimen by observing the inter-dose plasma exposure when the dose is increased or decreased [35]. It can assess complex clinical scenarios, such as potential DDIs due to variations in metabolism and transporters over time, PK in special populations with significant physiological differences, bioequivalence, food effects and pharmacogenomics [12]. PBPK might be valuable in assessing complex DDGIs and an increasing number of these models have been published recently [22,48]. They can be used during drug development to predict the vulnerability to DDIs in different genotypes groups and they allow bridging healthy adults to special populations and developing/testing hypotheses for unexpected clinical findings without clinical studies [22,49]. PBPK models have become increasingly prevalent to help prevent DDIs and ADRs but they may also help predict drug-disease interactions [12,22]. This thesis gives the example of the successful prediction of a complex drug-drug-disease-

interaction by PBPK modeling and it could thus be a key assistance in individualizing drug therapy regimens in choosing the optimal dose, frequency and route of administration [12,50].

However, the use of PBPK modeling for prediction is restricted by the partial knowledge of physiology and relevant PK mechanisms; the incomplete understanding of IVIVE for specific processes; and the limited *in vivo* relevant compound data for model confirmation [51]. PBPK modeling is a « bottom-up » approach and the lack of sufficient *in vitro* and *in vivo* data may be limiting factors [49]. Data are still needed to implement predictive systems into clinical practice, and knowledge on CYPs polymorphisms is not enough to predict DDIs solely [22]. As demonstrated in this thesis, some interactions are complex and depend on several factors, most being unknown. Performing IVIVE is therefore challenging in this context and prediction should ideally reach the same range as bioequivalence range. Indeed, the predicted value of PBPK model is validated between a 50-200% range of the observed value while the bioequivalence range is between 80-125%. Therefore, the PBPK validated range is currently too wide for models to be applied directly to patients. Moreover, the matrix must be considered, as PBPK modeling uses plasma, and a correction factor must be applied to compare simulation when observations are done in another matrix.
Health agencies have recognized the potential benefits offered by PBPK modeling but its direct application in risk assessment and public health decisions is still limited due to low confidence in model capabilities and uncertainties [52]. Nevertheless, PBPK, and more broadly MIPD, can improve health outcomes, decrease medication-related harm and reduce economic burden by individualizing treatment [46].

9.2 Conclusion

Phase I, II and III clinical studies do not include enough patients and have too restrictive inclusion and exclusion criteria to detect rare ADRs or efficacy issues in particular contexts. Pharmacovigilance through post-marketing studies and databases allows to generate real-world data, and drug administered to a larger number of patients may not have a profile as favorable as initially announced at the time of marketing authorization. New technologies bring the advent of Big Data and artificial intelligence, transforming how individual patient data are generated and processed. This provides increasing knowledge on variables that influence drug response. The detection and understanding of intra- and inter-individual variabilities through pharmacogenovigilance enrich precision medicine.

DMET genotyping is useful and readily available in clinical practice. Multiple DGIs have been already identified. However, precision medicine should not be based solely on pharmacogenomics. Indeed, non-genetic factors (environmental, physiological and pathophysiological factors) may induce phenoconversion, leading to a wrong prediction of DMET activity from genotype. Phenotype is dynamic, allowing the measurement of DMET activity at a given time and cocktail approaches are useful to assess multiple DME simultaneously. Phenotyping tests are shifting towards measuring endogenous compounds thanks to the progress that is being made in metabolomics.

The interactions between genetic and non-genetic factors are complex and genotype had an interdependency with phenotype. Indeed, the impact of non-genetic factors is dependent on the baseline activity of DMET in terms of magnitude, potency, and time-course. Henceforth, DDGDIs must be considered to be as close as possible to reality. This is how the individualization of treatments will be accurate and beneficial for the patient. Nevertheless, much remains to be done because it is assumed that several genetic variants and environmental factors are still unknown.

Today, several *in silico* approaches have shown utility in assessing clinical scenarios that become increasingly complex. For instance, PBPK could assist in individualizing drug therapy by managing complex clinical scenarios through the integration of all the known variables constituting an individual. Furthermore, the emerging integration and prediction of PD parameters are promising to individualize treatment.

Even though challenges remain regarding the integration of precision medicine in clinical practice, public health, regulation, drug development, pharmacovigilance and education, these developments will ultimately lead to an improvement of patient's safety and quality of care.

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Chapter 10: Annexes.

Research article 5: Snapshot of proton pump inhibitors prescriptions in a tertiary care hospital in Switzerland: less is more?

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RESEARCH ARTICLE

Snapshot of proton pump inhibitors prescriptions in a tertiary care hospital in Switzerland: less is more?

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Abstract

Background Proton pump inhibitors are among the most widely prescribed drugs in the world, but more than half of the indications for prescription are unjustified. The misuse of this therapeutic class has heavy consequences such as additional health costs, adverse drug reactions following long-term use and gastric acid rebound when the proton pump inhibitor is discontinued. *Objective* The overprescription of proton pump inhibitors is therefore becoming a public health problem, which led us to evaluate their use within the Geneva University Hospitals. *Setting* Patients hospitalized in two divisions of the department of internal medicine of the Geneva University Hospitals on a single day. *Methods* This is a register-based cross-sectional study and it collected data about the prescription pattern of proton pump inhibitors by consulting the electronic records of patients included. *Main outcome measure* To determine if the proton pump inhibitors prescription is made according to the market authorization and the available guidelines. *Results* Hundred-eighty patients were included. 54% of patients were on proton pump inhibitors, 29% of whom had their treatment initiated at hospital. Of the indications for treatment, 72% were not justified and 63% of the justified indications did not have an adequate dosage. Therefore, in all patients with a proton pump inhibitor at hospital, only 11% had a justified indication with an adequate dose. Finally, 87% of known home prescriptions were renewed on admission and among them, 71% did not have a justified or possibly justified indication according to the guidelines. *Conclusion* Indication for treatment inside the hospital was not justified in 72% of patients and only 11% had a justified indication with an adequate dosage. Precise guidelines with evidence-based indications and adequate daily doses would help to correctly prescribe proton pump inhibitors. Moreover, patients should benefit from a thorough evaluation of their treatment.

Keywords Drug-safety · Pharmacoepidemiology · Prescribing · Proton pump inhibitors · Public health · Switzerland · Tertiary care centers

Impact of findings on practice statements

- Around 70% of the renewed prescriptions for proton pump inhibitors on admission did not have a justified indication.
- As many as 72% of the hospitalised patients had an unjustified indication for a proton pump inhibitor.

Introduction

Proton pump inhibitors (PPIs) were introduced to the market at the end of the 1980s [1] and are currently among the most widely prescribed treatments worldwide [2], with

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more than 60% of them prescribed for an unjustified indication [3] in US ambulatory settings. The chronic use of PPIs, the difficulty in stopping the treatment due to gastric acid rebound symptoms [4], their effectiveness and their very good short-term tolerance, leading to an estimated compliance of 71%, explain the magnitude of their prescription [5].

In the US among others, PPIs are therefore associated with a problem of overuse [6], despite clearly established prescription guidelines, evidence of adverse effects in the case of prolonged intake and additional cost incurred [7]. In Switzerland, there are similar concerns and indeed the better usage of PPIs is part of a national campaign for the reduction of PPI overuse, the "Smarter Medicine" campaign, set up by the Swiss Society of Internal Medicine [8].

A prospective study performed in a department of internal medicine in France showed that only 33% of the indications were within the scope of the marketing authorization. Amongst the 67% of off-label prescriptions, 69% of PPIs were given as a prevention against potential gastrointestinal lesions when an antiplatelet drug, a corticosteroid or an anticoagulant was prescribed and this in subjects without any risk factor for digestive haemorrhage [5]. This study also found that PPI prescription increased significantly with age, with more than half of the patients aged 70–79 years old receiving a PPI [5].

The most common misuse of PPIs is their use for the prophylaxis of stress ulcers outside the intensive care unit (ICU) [9]. A study conducted in the US on non-ICU patients showed that 79% did not have any risk factor for a stress ulcer and therefore received an unjustified PPI prophylaxis [10].

The misuse of PPIs can also be measured by the use of an inappropriate dosage. The recommendations for esomeprazole as a prophylaxis are 20 mg per day in almost all indications, while the 40 mg daily dose is commonly prescribed, without any demonstrated benefit [11].

Whereas short-term use is generally well tolerated, the over-use of PPIs may also be related to the lack of awareness of the harmful effects of their long-term use. Prolonged PPI intake is associated with an increased total risk of fractures [4, 12, 13], although a small recent study shows that long-term PPI use does not appear to promote changes in bone mineral density and strength that could predispose to fractures [14]. Also, a recent retrospective cohort study does not evidence an association between PPI use and fracture risk among older adults [15]. PPI intake is also associated with an increased total risk of nosocomial [16] and community-acquired pneumonia [17], and intestinal infections [18, 19] by *Clostridium difficile*, *Shigella*, *Campylobacter* and *Salmonella* [20]. These intestinal infections could be explained by the fact that PPIs modify the gut microbiota as shown in several studies [21, 22]. A recent study on over-the-counter (OTC) PPI use shows no association with pulmonary and intestinal infections. However, in this study, the PPIs were taken at a lower dose and for a shorter period of time compared to other studies [23]. In addition, PPIs are among the drugs commonly associated with the occurrence of tubulointerstitial nephritis [24], which may lead to acute renal failure [25]. Long-term treatment with PPIs may also induce deficiencies in vitamin B12, iron, and magnesium [26, 27]. Moreover, a population based study demonstrated that long-term PPIs use, even after *H. pylori* eradication therapy, is associated with an increased risk of gastric cancer [28], though this study was not in line with a previous meta-analysis [29].

PPI prescription can also lead to drug interactions. The absorption of other drugs may be influenced by the increased gastric pH associated with the intake of PPIs [4], resulting in either an increase but also a decrease in the absorption of these concomitant drugs. In addition, several PPIs have inhibitory effects on different cytochromes, in particular on CYP2C19 and 3A4 and on the P-gp transport system [30]. From a pharmacodynamic point of view, some authors have raised concerns about the prescription of PPIs with aspirin, claiming a reduced cardioprotective effect of aspirin. This could be due to a reduced absorption of aspirin but also to a reduced platelet response through increased serum thromboxane B2, and therefore also endogenous thromboxane A2 [31].

A database study from 2016 demonstrates that the PPI use in UK general practices increased between 1990 and 2014 and that 60% of long-term users did not attempt to discontinue or step down the dose [32]. These data suggest that the re-evaluation of the adequacy of the PPI treatment was not done although patients should benefit from a reconsideration of the usefulness of their treatment on a regular basis. This study highlights the need of a "Smarter Medicine" and the validity of the campaign mentioned above. The authors advance that by improving withdrawal strategies, a reduction of the costs and the occurrence of adverse effects should be possible [32].

Esomeprazole is the most widely used PPI at the Geneva University Hospitals. The hospital benefits from attractive prices, but this hospital prescription influences the community prescription, participating in an estimated extra cost of 30.3 million euros between 2000 and 2008 [33]. In Switzerland, esomeprazole is not an OTC so PPIs intake comes from medical prescription.

Aim of the study

The aim of our prospective study was to understand the PPI prescription in the department of internal medicine of our hospital and in particular to determine if the PPI prescription is made according to the market authorization and the available guidelines.

Ethics approval

All procedures performed in our study were in accordance with the ethical standards of the regional research ethics committee of the canton of Geneva (No. 2016-00580) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Methods

Study design and setting

This study was a register-based cross-sectional study. The data was collected by consulting the electronic records of patients hospitalized in two divisions of the department of internal medicine of the Geneva University Hospitals (division of general internal medicine and division of general medical rehabilitation) for a total of 11 wards. On a given day, the electronic records of all patients of one of the wards were consulted and data collected by using the computerized patient record system of the Geneva University Hospitals (DPI®). The whole data collection (all patients of all the wards) was done in the same week, meaning that all the electronic records of a single ward were looked at on the same day but the whole data collection was done over a week. All patients' data was anonymised.

Inclusion criteria

Patients over 18 years old and hospitalized in either the division of general internal medicine or the division of general medical rehabilitation on the day of the study.

Primary and secondary outcomes

The primary outcomes was the proportion of patients receiving a PPI at hospital and at home.

The secondary outcome was the adequacy of the indication for a PPI in patients receiving this treatment. Evaluation of the adequacy was done following Table 1.

This table was built taking into account the National Institute of Health and Care Excellence (NICE) guideline "Gastro-oesophageal reflux disease and dyspepsia in adults: investigation and management" [34] for the justified indications and the Swiss Summary of Product Characteristics of the PPIs for the possible justified indications that were not already mentioned in the NICE guideline. We chose the NICE guideline because of the absence of a European or a Swiss guideline. These guidelines have been used to develop internal recommendations in our hospital and so we expected that the Geneva University Hospitals prescribers would follow them.

Statistical analysis

Data management

The data was collected anonymously and consisted of collecting "patient" data (gender and age) and "PPI" data (indication, international nonproprietary name (INN), dosage and route of administration, if the treatment was initiated on admission and the evaluation of the adequacy of the treatment).

Statistical strength and data analysis

This was a cross-sectional study aimed at characterizing the PPI prescription for hospitalized patients on a specific day. There was no hypothesis about the expected value of subjects. The analysis consisted of descriptive statistics separated into several parts, such as "PPI prescription at the hospital" and "PPI treatment at home".

Results

The total of patients screened and included in this study was 180 and the flowchart is shown in Fig. 1.

The average patient age was 65.7 years old and the distribution of men and women was respectively 53% (N = 95) and 47% (N = 85).

For patients for whom home treatment was known (N = 171), 54% (N = 93) did not have a PPI at home and 46% (N = 78) of patients were already treated at home. Home treatment was undocumented for 9 patients. Among this overall population, 54% (N = 97) were treated with a PPI at the hospital. The average age of these patients was 64.4 years. The percentages of men and women was respectively 48% (N = 47) and 52% (N = 50). No gender or age differences were seen between patient treated by a PPI or not.

Of these 97 patients treated with PPI at the hospital, 29% (N = 28) of patients had their PPI treatment initiated at hospital. The most common PPI prescribed was

Table 1 Classification of indications

Possibly justified treatment with esomeprazole (Swissmedicinfo.ch)
40 mg/d
Prophylaxis of ulcer recessions associated with *Helicobacter pylori* (*20 mg twice daily for 7 days*)
20 mg/d
Long-term prophylaxis of reflux oesophagitis recurrence
Treatment of symptomatic reflux after disappearance of symptoms, if no NSAID or emergence of new disorders
Justified treatment with esomeprazole (NICE)
> 40 mg/d
Hypersecretion, including Zollinger-Ellison syndrome and idiopathic hypersecretion (*40 mg twice daily at the beginning and possible increase at 80* (− 120) *mg twice daily*)
i.v: Treatment and prevention of new haemorrhages of a documented gastric or duodenal bleeding ulcer (*80 mg in bolus by fast infusion during 30 min then 8 mg/h during 72 h*)
40 mg/d
Treatment of severe esophagitis (*8 weeks*)
Maintenance treatment for patients with severe esophagitis (*if maintenance treatment fails, change with another PPI at full-dose or high-dose*)
H. pylori eradication therapy (*2 × 20 mg for 7 days*)
Prevention of new haemorrhages of a gastric or duodenal ulcer after treatment with esomeprazole intravenous (*4 weeks*)
20 mg/d
If taking a needed NSAID while diagnosed peptic ulcer (*8 weeks*)
Patient with NSAID with peptic ulcer diagnosed (*8 weeks*)
Not investigated dyspepsia (*4 weeks*)
Treatment of gastroesophageal reflux (*4–8 weeks*)
Patient with dilation of the oesophagus following stenosis (*long term*)
Peptic ulcer treatment for patients who are *H. pylori* negative and who are not on NSAIDs (*4-8 weeks*)
Treatment of functional dyspepsia if *H. pylori* excluded and symptoms persist (*4 weeks*)

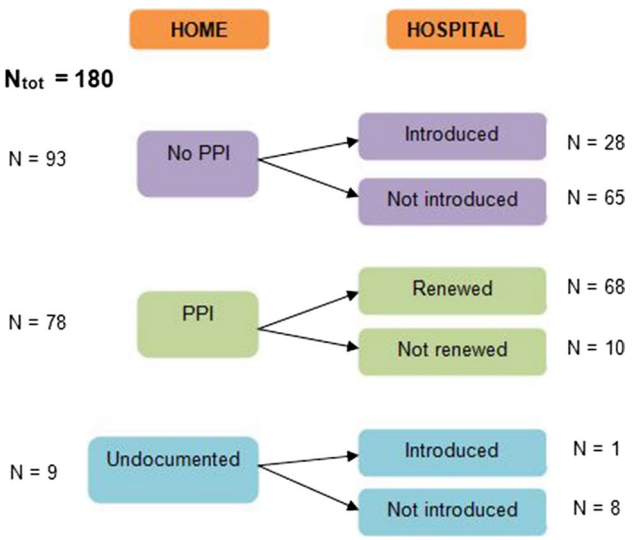

Fig. 1 Diagram of the distribution of patients included

esomeprazole (97%, N = 94), followed by lansoprazole (2%, N = 2) and pantoprazole (1%, N = 1). The predominant route of administration was oral at 94% (N = 91), followed by intravenous (4%, N = 4), and perfusion (2%, N = 2). The predominant dosage of esomeprazole was 40 mg/d in 50% of cases (N = 47). Other dosages were 20 mg/d in 35% (N = 33), 80 mg/d in 12% (N = 11), 8 mg/h in 2% (N = 2) and finally 120 mg/d in 1% (N = 1) of patients.

For these same 97 patients, regardless of the dosage, 72% (N = 70) of the indications were unjustified, 4% (N = 4) were possibly justified and 24% (N = 23) were justified in the patient's files. Of these justified and possibly justified indications, 25% (N = 1) and 44% (N = 10) respectively had an adequate dose. Therefore, in all patients with a PPI at hospital, only 11% had a justified and possibly justified indication with an adequate dose. The most frequently reported unjustified indication was prophylaxis of bleeding when the patients received also an NSAID, an anticoagulant or an antiplatelet drug with a frequency of 24% (N = 17).

Regarding patients that did not have a PPI at home (N = 93), the same 28 (30%) had one initiated on admission. Of these 28 patients, 79% (N = 22) of treatments implemented at the hospital had no valid indication, whereas only 21% (N = 6) had a justified or possibly justified indication. Of the 65 patients that did not have a treatment initiated at the hospital, 3% (N = 2) would have had an indication to receive one. The population which didn't need a PPI and

didn't have a PPI represents 35% (N = 63) of the total of patients screened.

Regarding patients that did have a PPI at home already (N = 78), esomeprazole was the most prescribed (70%, N = 54), followed by omeprazole, pantoprazole and lansoprazole with a rate of 20% (N = 16), 9% (N = 7) and 1% (N = 1) respectively. Oral route was the only one used (100%). Regarding the dosage, 40 mg/d was prescribed in 60% (N = 32) of esomeprazole prescription cases, 20 mg/d in 35% (N = 19) and 80 mg/d in 5% (N = 3). Of these, 13% (N = 10) saw their prescription stopped on admission to hospital. In most cases, documentation available in the admission letter did not allow classifying the home prescriptions as justified, possibly justified or unjustified.

Finally, 87% (N = 68) of known home prescriptions were kept on admission and among them only 29% (N = 20) were valid because the indication was justified or possibly justified according to the guidelines. It also means that 70% (N = 68) of hospital PPI prescriptions (N = 97) come directly from home but only 21% (N = 20) are for valid indications.

Discussion

The prevalence of PPI prescription in our hospital is high with more than half the patients on PPI treatment. This is in line with other studies, conducted in the US and in Europe, where the prescription rate can reach up to 80% [7, 35]. A study conducted in a Qatari hospital highlighted that the prescription of acid suppressive therapy concerned 53% of patients and the proportion of PPI and histamine 2 antagonists was 89% and 11% respectively [36]. Moreover, 29% of the prescriptions in our study were new prescriptions initiated on admission to the hospital. This is slightly lower than percentage reported in Villamanan et al. study, where 49% of patients had a treatment initiated on admission [35].

The most common used PPI at our hospital is esomeprazole with 97% of patients receiving this drug. This is due to the implementation in our hospital of a restrictive drug formulary aimed at minimizing acquisition costs and limiting the number of medications available in our hospital. Esomeprazole is listed in our formulary and is therefore the reference drug in its therapeutic class. However, the dosage of 40 mg/d, prescribed in 50% of our patients, has no reasonable explanation other than aiming at an optimal efficacy of a medication with a favourable short-term risk–benefit ratio, from prescribers that prefer to avoid gastric complications whilst ignoring the long-term adverse effects.

Regarding adequacy of the prescription, 89% were unjustified in the patient file when taking into account the indication and the daily dose, with 72% having an inadequate indication and a further 17% having a justified or a possibly justified indication but with an inadequate daily dose.

A study conducted in a French hospital showed that the prescriptions that were not justified as mentioned in the market authorisation of the PPIs were as high as 74% [5]. The study conducted in a Qatari hospital also highlighted that only 34% of patients had a justified prescription, PPI and anti-histamine 2 antagonists together [36]. The high rate found in our study can be partly explained by the fact that the Summary of Products Characteristics for PPIs in Switzerland is unprecise and less restrictive than the indications considered justified in our study but shows nevertheless that correct indication and correct dosage are a crucial problem in the prescribing of PPIs. Moreover, when it comes to patients that have a treatment initiated in our hospital, again 89% receive a PPI for an unjustified indication. A study conducted in Spain reports a rate of only 36% [36], but again this can be explained by the fact that they were less restrictive on the indications than we were in our study.

Interestingly, nearly half the patients already had a PPI in their treatment on admission to the hospital with 70% of them treated with esomeprazole and nearly 60% taking a 40 mg/d dosage. This confirms the apparent impact of the hospital on the community due to the continuity of care. This influence was demonstrated by a study conducted at the Geneva University Hospitals which showed the extra cost of this continuity of care, because the generics of esomeprazole didn't exist at the time of the study, between 2000 and 2008 [37]. It also demonstrates that general practitioners are prone to over-prescribing this therapeutic class. Among our patients, nearly 90% saw their prescription of PPI continued on admission ignoring the fact that the indication was justified in only 29% of patients. The study conducted in the Qatari hospital found that the usage of acid suppressive medication could even worsen with a 12% of prescription rate before admission to a 53% during hospitalisation [36]. This is in contradiction with the opportunity that a hospital has to review properly the treatment of the patients. Our study did not aim to quantify the prescription at discharge but the Qatari study showed that this is also a problem. In their study, 54% of PPIs with a non-justified prescription were still prescribed upon discharge and this rate was still of 50% six months after discharge [36]. Prescriptions seem not to be reassessed at admission and at discharge and this is a vicious circle which influences prescription in the community and leads to long-term treatments.

A possible explanation of the overuse of PPIs could be the occurrence of rebound acid hypersecretion (RAHS) [4]. RAHS is defined as an increase in gastric acid secretion above pre-treatment levels after anti-secretory therapy. It leads to acid related symptoms such as heartburn, acid regurgitation, and dyspepsia. The acid rebound hypersecretion seems to be caused by an increased acid secretion by proton-pump stimulation due to a compensatory gastrin release and an hypertrophy of enterochromaffine-like cells through

an increased level of chromogranin A [37–40]. A study from 1996 reports that the gastric acid secretory capacity was increased by 22% 14 days after discontinuation of a 3-month course of omeprazole 40 mg/d [38]. Some studies demonstrate that the discontinuation of PPI is a success in only 19 to 27% of long-term PPI users, after 12 months [41, 42]. Also, discontinuation is most successful when patients have no gastro-oesophageal reflux disease [42]. The success of PPI discontinuation in patients with inappropriate indications is a major health concern in the light of the number of patients receiving an inappropriate and long-term prescription and the associated excessive health care costs [43, 44]. The discontinuation of PPI is not easy. Bjornsson and al. [41] suggested to use a gradual discontinuation of PPI in order to prevent the consequence of the acid rebound effect. However, they failed to show a different rate of discontinuation between patient in the group with abrupt discontinuation and in the group with gradual discontinuation. The authors conclude that a gradual weaning of the PPI might be useful only in the rare patients with hypergastrinemia. At the present time, the level of evidence is not sufficient to recommend a method of PPI withdrawal.

Another approach against PPI overuse was evaluated in a prospective study. The approach was a multi-approach strategy through an audit and feedback method, the implementation of a usage guideline for medical inpatients, the diffusion of a logarithmic chart on the proper usage of acid suppressive medications for medical inpatients from admission through to discharge and the participation of clinical pharmacist in the multidisciplinary rounds [45]. This approach allowed decreasing the inappropriate use of acid suppressive therapy. There was a 51% ($p < 0.0001$), a 62% ($p < 0.0001$) and a 67% ($p = 0.0008$) decrease of inappropriate use during admission, at discharge and at the two months follow-up visit, respectively [45].

Our study has some limitations. First, due to the study's methodology, our analysis was limited by the quality of the documentation of the patients' records in the computerized patient record system of our hospital. Secondly, often, at times, assumptions had to be made about the indication because this was not explicitly documented. It was by studying concomitant medication, gastroenterological examinations and medical history that indications, valid or otherwise, were presumed. Finally, we did not collect any data on PPI prescription at discharge that would have given us an idea of the community prescription.

Conclusion

Our study, conducted in a tertiary care hospital in Switzerland, highlights the problems surrounding the prescription of PPIs. These problems are multifactorial and the solutions should therefore be addressed from different angles. Improving prescribers' awareness of the over-use of PPIs and the long-term adverse effects as well as setting up guidelines in our hospital would be a first step in minimizing the overuse of this therapeutic class. Guidelines built on evidence-based indications and adequate daily doses would help the prescribers, in the hospital and in the community, to adequately prescribe and reduce misuse of PPIs. Integration of a prescription assistance programme in our computerized patient record system could help to identify the correct indication and the correct dose and limit the length of prescription.

Also, when admitted to the hospital, patients should benefit from a thorough evaluation of their treatment according to their clinical utility and at discharge, reconsideration of the usefulness of the hospital treatment should also be considered.

Finally, education and patient awareness could also significantly reduce the use of PPIs, especially in the long term.

This study was a pragmatic real-life study. The medical structure in which it was conducted and the patient's characteristics should allow our results to be extrapolated to other general inpatient medical wards in Swiss teaching hospital. The Geneva University Hospital being one of the five university hospitals in Switzerland and the largest one, data from this study could be of value for other countries also.

All procedures performed in our study were in accordance with the ethical standards of the regional research ethics committee of the canton of Geneva (No 2016-00580) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Conflicts of interest The authors declare that they have no conflict of interest.

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Research article 6: Outcomes of drug exposition during pregnancy: Analysis from a teratology information service

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Full length article

Outcomes of drug exposition during pregnancy: Analysis from a teratology information service

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ABSTRACT

Objective: We aimed to characterize drug exposures during pregnancy where the outcome was known that had benefited from counselling through our Teratology Information Service (TIS) between 1994–2016.
Study design: This observational study analysed data collected through the drug exposures during pregnancy counselling. Data was analysed descriptively.
Results: Data from a total of 1'374 pregnant women were collected. Mean age was of 32 years. These women were exposed to more than ten drugs in 1.4 % (N = 19) of cases, with a mean drug intake of two. Analysis of the drugs altogether (N = 3'129) showed that FDA Pregnancy Category C drugs represented 42.9 % (N = 1'342) of drugs and ATC code N (nervous system) represented 36.4 % (N = 1'138). The onset of drug exposure was during the first trimester of pregnancy in 95.1 % (N = 2'982) of patients. Regarding outcomes, the rate of induced abortion was 10.8 % (N = 151), of pregnancy complications was 11.2 % (N = 157) and of malformations was 4.5 % (N = 49).
Conclusion: Pregnant women counselled by our TIS take a mean of two drugs, ranging from one to 17. Drugs are from FDA Pregnancy Category C and ATC N drugs in most cases, 42.9 % and 36.4 % respectively. The rate of malformation of our cohort was of 4.5 %, close to the estimated spontaneous rate of malformation. This data gives a reassuring aspect of drug exposure in pregnancy but takes into account the outcome at birth only.

1 Introduction

Many pregnant women are exposed to drugs, either occasionally or for a prolonged period, due to acute or chronic illnesses. The percentage of women exposed during pregnancy varies according to age at the time of pregnancy, ethnicity, level of education, health insurance system and geographic region [1]. Half of all pregnancies are unplanned, making it common for women to be exposed involuntarily at the beginning of their pregnancy [2].

In a prospective cohort study of nulliparous women followed since the first trimester, 73.4 % of women took a drug during their pregnancy with 55.1 % taking at least one drug during the first trimester, the critical period for development [1]. Polypharmacy is defined as taking more than five drugs and the same study showed that this was the case for 13 % of pregnant women [1]. In another study, women received an average of 5.2 medications in the first trimester, 7.1 in the 2nd and 6.6 in the 3rd trimester [3].

In a 2011 meta-analysis, French women were those who were the most exposed to drugs during pregnancy with a mean of at least 10 different drugs which was far above estimates in all other countries included in the study (Netherlands, Germany, Norway, Denmark, Finland, Italy and the US) [4].

The most commonly prescribed drugs in the first trimester are those for the gastrointestinal system, followed by antibiotics and analgesics [1]. However, the most prevalent drug group among the consultations received by Embryotox (a centre for pharmacovigilance and counselling in embryonic toxicology located in Berlin) are the psychotropic drugs, representing 25 % of all drugs [2].

The knowledge of the risk associated with drug exposure during pregnancy has improved substantially since the thalidomide scandal 60 years ago. When a drug is marketed now, in vivo and in vitro studies estimate the risk associated with exposure during pregnancy. The International Council of Harmonization (ICH) provides recommendations (ICH S2 and S5) for the industry to

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highlight important factors for assessing the potential risk of a toxic effect in humans [5, 6]. However, clinical experience is still insufficient regarding the safety of drugs in pregnancy and often only epidemiological studies allow a risk assessment [2].

Adverse effects of drugs during pregnancy can be separated into two different categories, either teratogenic or foetotoxic. A teratogenic drug is defined as causing irreversible impairment to the newborn, affecting the organs during embryological development and thus causing birth defects. A foetotoxic drug is a drug that has a detrimental effect on foetal growth and organ function [7]. These effects can be due to the dose, the duration of exposure, the route of administration, the concomitant exposures, the period of exposure during pregnancy and potential genetic predispositions.

Since 2008, FDA does not recommend using the FDA risk categories anymore [8] and, in 2015, the FDA pregnancy risk classification system was replaced by the final Pregnancy and Lactation Rule [9]. From then on, FDA pregnancy risk categories also gradually disappeared from the Swiss Summary of Product Characteristics. The older FDA classification was as follows [8] :

- A: controlled studies in women do not show risk to the foetus in the first trimester
- B: animal reproduction studies do not show risk to the foetus but there are not controlled studies in pregnant women
- C: studies in animal show adverse effects on the foetus but there are not controlled studies in women
- D: there is positive evidence of human foetal risk, but benefits from use in pregnant women may be acceptable despite the risk
- X: studies in animal or human beings show foetal abnormalities and the risk of the use of the drug in pregnant women clearly outweighs any possible benefits

Knowledge of the potential adverse effects of drugs during pregnancy can help protect the mother and the baby. However, conversely, the overestimation of the associated risks may lead to withholding essential therapy, poor adherence, prescription of insufficiently studied drugs, invasive prenatal diagnostic tests or the recommendation to terminate a pregnancy [2]. A study compared pregnant women with an “average drug exposure” (teratogens and/or foetotoxic drugs excluded) and non-exposed or insignificantly exposed pregnant women and showed that the elective induced abortion rate (11 %) is higher in case of drug exposure while the rate of miscarriage and malformations were similar, with rates of 16 % and 3 % respectively [2]. The risk of major and minor malformations in the general population is estimated at 3–4 % and the aetiology of these is unknown in up to 70 % of cases [10]. Malformations due to drugs are thought to represent less than 2 % of this estimated risk [11].

Our Teratology Information Service (TIS) works by request for consultations by the physicians that follow pregnant woman exposed to a drug during pregnancy. These requests can come from the whole of Switzerland but most of them are from the French-speaking part.

Our TIS has been a dynamic and constantly improving centre over the last quarter of a century. During the last three years of the study, our TIS had a mean of 165 consultation requests per year. Follow-up for drug exposure during pregnancy is directly requested to the physician six weeks after the scheduled date of delivery. During the last three years of the study, follow-up was obtained in 88 % of cases.

The aim of our study was to describe the outcome of drug exposed pregnancies that we encountered among the consultations of our TIS, during more than 20 years of counselling. The TIS is part of the Division of Clinical Pharmacology and Toxicology of the Geneva University Hospital, Switzerland.

2 Methods

This study is a descriptive prospective cohort study conducted between 1994 and 2016. This study was approved by the research ethics committee of the canton of Geneva (No 2017-00625). The data were collected prospectively in the computerized database of clinical pharmacology consultations, the paper archives and the Excel files maintained since 1994 that collected the follow-up of the pregnancies.

2.1 Inclusion criteria

Consultation reports from the Division of Clinical Pharmacology and Toxicology of the University Hospital of Geneva between 1994 and 2016 that were on drug exposure during pregnancy and for which the outcome of the pregnancy was known.

2.2 Non-inclusion criteria

Consultation reports from the Division of Clinical Pharmacology and Toxicology of the University Hospital of Geneva between 1994 and 2016 that were on drug exposure during pregnancy but for which the outcome of the pregnancy was unknown or consultations requested before the pregnancy and resulting in no drug exposure during pregnancy.

2.3 Primary and secondary outcome

To characterise drug exposures during pregnancy among women included in our counselling service between 1994–2016. To provide a reasonable approximation of the malformation rate during pregnancy exposed to drugs and compare it to that found in the general population. To provide a reasonable approximation of the complication rate that occurred at delivery, such as preterm birth, intrauterine growth restriction (IUGR), respiratory distress syndrome, withdrawal and others (hypoglycaemia, icterus, oligoamnios..etc).

2.4 Statistical analysis

2.4.1 Data management

The data for the analysis were evaluated anonymously and consisted of:

- Maternal data : age, medical history
- Specific data concerning pregnancy : date of the last menstruation, estimated date of delivery
- Pregnancy outcome : delivery (premature or not), spontaneous abortion, elective induced abortion
- Newborn data: date of the birth, weight, Apgar score, clinical status (premature, birth defect, withdrawal symptoms . . .)
- Drug: International Nonproprietary Names (INN), ATC code, dose, route of administration, date of beginning and end of treatment

Drugs were classified according to the old FDA classification because it was the system used at the time when the drug exposures during pregnancy of our cohort happened. It also allows a classification of the risks of each drug. Moreover, the FDA risk categories are known to everyone and this makes it clearer to fully appraise the results.

2.4.2 Statistical strength and data analysis

This is a descriptive cohort study aimed at describing the outcome of drug exposure during pregnancy. There was no

hypothesis about the expected number of subjects. The analysis consisted of descriptive statistic data.

3 Results

A total of 1374 pregnant women were included in this study, the average age was 32 years old ranging from 14 to 48 years old. The most represented age group was the 30–34 age group (31.7 %). Half of the women were exposed to a single molecule (49.1 %, N = 674) with a maximal exposure of 17 molecules (N = 3) (Fig. 1).

The total of all drugs taken during these 1′374 pregnancies was of 3′129 as there was often more than one drug taken as mentioned above. Most counselling were for FDA class C or of unknown FDA pregnancy category with 42.9 % (N = 1′342) and 28.3 % (N = 886) respectively. FDA class X pregnancy category accounted for 4.9 % (N = 154) of the total of drugs (Fig. 2).

Most of the requests concerned drugs of the nervous system (ATC class N) and anti-infectious drugs (ATC class J) with 36.4 % (N = 1'138) and 17.9 % (N = 559) respectively of the total of all pregnancy medications (N = 3′129) (Fig. 3). Sedatives (e.g. alprazolam, zolpidem, bromazepam, midazolam . . .) and antidepressants/antipsychotics (e.g. clomipramine, amitriptyline, fluoxetine, citalopram, quetiapine . . .) accounted for 37.6 % (N = 428) and 30.6 % (N = 348) of drugs in the ATC class N, respectively (N = 1′141). Among the ATC class J (N = 559), antibiotics accounted for half of the requests (50.1 %, N = 280).

Drug therapy (N = 3′129) was almost always started in the first trimester of pregnancy (95.1 %, N = 2′982).

Outcomes were first analysed taking into account the total number of embryos/foetuses, that is, 1′374 pregnancies plus 22 twins of 22 twin pregnancies (N = 1′396).

On the whole cohort of embryos/foetuses (N = 1′396), there were 66.5 % normal neonates (N = 929) and 11.2 % neonates with complications (N = 157) for a total of 77.8 % live births (N = 1′086). Embryo/foetus/neonate deaths represented 14.6 % (N = 159) and could be further separated into 112 spontaneous abortions (8.0 %), 31 therapeutic terminations of pregnancy (2.2 %) and 16 other foetal deaths (1.1 %) (i.e. in-utero and neonatal deaths and ectopic pregnancies). Finally, 151 (10.8 %) pregnancies ended in an elective induced abortion.

Further analysis was done taking into account the number of live births.

Of all neonates (N = 1′086), 9.9 % (N = 107) were premature, this rate being higher in the 22 twin pregnancies with a rate of 68.2 % (N = 15) premature deliveries.

Of all neonates (N = 1′086), the rate of at least one complication was 14.5 % (N = 157) with 4.5 % (N = 49) being malformations (minor, major or chromosomal/genetic disorders). The different complications and the corresponding proportions are detailed in Table 1.

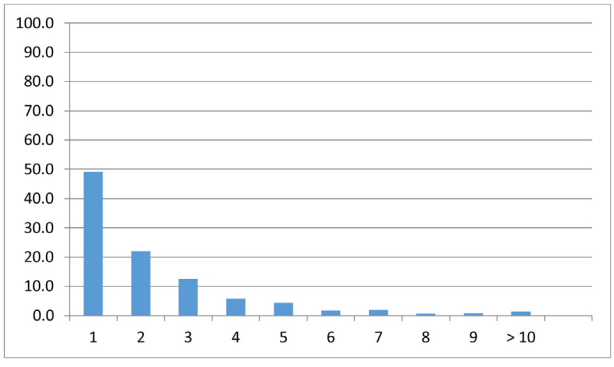

Fig. 1. Distribution of the number of drugs taken by each pregnant woman.

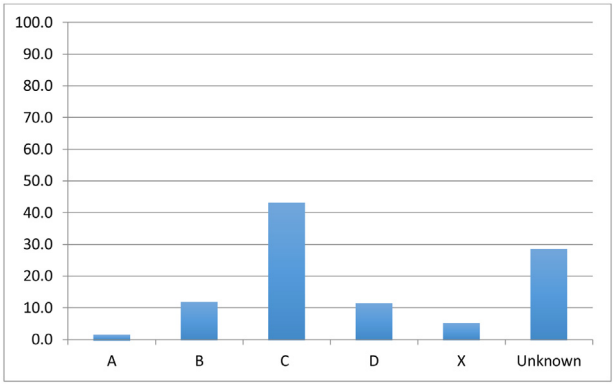

Fig. 2. Distribution of drugs according to the FDA classification.

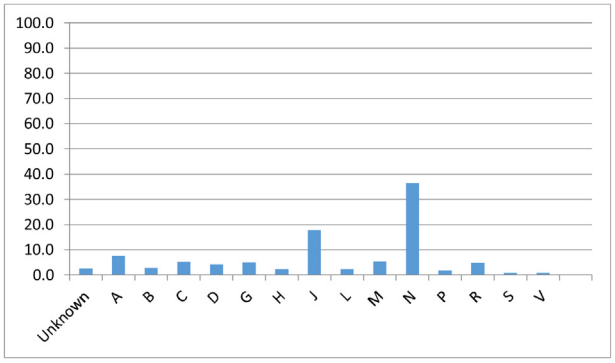

Fig. 3. Drug Distribution by ATC Class.

For intrauterine deaths and therapeutic terminations of pregnancy, one (trisomy 21) and four (trisomy 21, trisomy 18, spina bifida, major cardiac malformation) malformations were documented respectively. These malformations are not included in the calculation because we wanted to evaluate the impact of drug exposure during pregnancy on living malformations. All of the malformations encountered in the 49 neonates with malformations are detailed in Table 2.

The 49 malformations observed were after exposure to a total of 114 drugs, women often taking several drugs. Drugs from ATC class N (nervous system) represented 61 of these drugs and were present in 31 malformations. Drugs from ATC class A (alimentary tract and metabolism, e.g. antidiabetics, antiemetics) represented ten of these drugs and were present in five malformations. And finally, drugs from ATC class C (cardiovascular system) represented 10 of these drugs and were present in four malformations.

Among the 33 exposures to isotretinoin during pregnancy collected, there were twenty (60.6 %) therapeutic terminations of pregnancy, though none had a documented malformation, one ectopic pregnancy, two spontaneous abortions, one premature birth and nine normal outcomes (27.3 %).

Among the 15 exposures to valproate during pregnancy collected, there was one neonate with a malformation (absence of distal phalanx and syndactyly), four (26.7 %) elective induced abortions, one IUGR, one spontaneous abortion and eight (53.3 %) normal outcomes.

Among the 37 angiotensin-converting-enzyme (ACE) inhibitor and angiotensin II receptor blocker (ARB) exposures during pregnancy collected, there were twelve (31.6 %) premature births, fourteen (36.8 %) normal outcomes, one IUGR, five elective induced abortion), three spontaneous abortions, one renal insufficiency (resolving after discontinuation of treatment) and one malformation

Table 1
Details about complications.

Complication	Number (N)	Percentage	Expected rate
Neonates with malformation	49	4.5 %	3.4 %[1]
Intrauterine growth restriction	36	3.3 %	3–7 %[2–3]
Respiratory distress syndrome	21	1.9 %	Consistent variability between and among continent[4]. E.g. 17.9 per 100'000 person-years in Europe. Under-recognition incidence: 40–50 %[4]
Withdrawal	26	2.4 %	Variability between states and rural or urban infants[5]. 8.0/1000 hospital births in in 2014 in the US[6].
Other (e.g. hypoglycemia, icterus, hypernatremia, oligoamnios . . . etc.)	25	2.3 %	/
Total	**157**	**14.4%**	**/**

[1] https://www.entis-org.eu/.
[2] Vandenbosche RC, Kirchner JT. Intrauterine growth retardation. Am Fam Physician. 1998 Oct 15;58(6):1384-90, 1393-4.
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(clubfoot). Among the premature births, there were four births with complications (one respiratory distress syndrome, one withdrawal and two hypoglycemia and two cardiac malformations.

In most of these cases, other drugs were also taken concomitantly.

4 Discussion

It has been estimated that only 10 % of drugs marketed since 1980 have sufficient data on efficacy and safety during pregnancy, so the use of drugs during pregnancy is a decision based on the benefit/risk balance [12]. In addition to the risks associated with drug exposure, there are physiological changes during pregnancy that can lead to changes in the pharmacokinetic of the drugs [12]. For example, there is an increase in the amount of water in the body, of the volume of blood, of the volume of distribution and of the renal blood flow. Intestinal motility is impaired, liver enzyme values, albumin and plasma pH are reduced [12]. All of these can alter the foetal exposure by potentially increasing the plasmatic concentration of active drugs or metabolites and hence the teratogenic potential that can be dose-dependent.

Regarding the number of drugs taken, half of the women in our cohort were exposed to only one drug but several had multiple prescriptions, the number of drugs reaching 17 at the most. This is in line with other observations of drug exposure during pregnancy [1–4].

We chose to analyse our data using the old FDA classification because the requests at the moment of our study were tightly related to the FDA classification of the drug. Indeed, most reports of drug exposure during pregnancy of our cohort are for FDA class C or of an unknown class, so for drugs where either a risk exists in the animal but has not been tested in humans or where there is no data available. This reflects the concerns of doctors that ask for teratology counselling because information on these drugs is scarce or inexistent. Therefore, our analysis may underestimate the number of drugs from other classes taken by pregnant women, as practitioners did not ask counselling for drugs known to be harmless or conversely, drugs with known harms but required for treating the pregnant women.

In our cohort, regarding ATC class N (nervous system), antidepressant/antipsychotic were among the most commonly reported drugs taken during pregnancy. This can be explained in part by the fact that depression is common during pregnancy with a prevalence ranging from 7.4%–12.8 %, depending on the trimester [13]. There are three main categories of antidepressants (tricyclic, selective serotonin reuptake and serotonin/noradrenalin inhibitors) but the most extensively documented are SSRIs. Neonates exposed in-utero to SSRIs have an increased risk of morbidity and some small studies have demonstrated an increased risk for prematurity, admission to special neonatal care, poor neonatal adaptation including respiratory difficulties, low Apgar score, hypoglycaemia, feeding difficulties and cerebral excitation [14]. However, these effects are generally transient and SSRIs are considered to be the first choice of antidepressants when depression in pregnancy needs to be treated [14]. A systematic review and meta-analysis concluded that women who received SSRIs during pregnancy had a significantly higher risk of developing preterm birth compared with controls and this remained significant even when comparing depressed women only [15]. Moreover, an other systematic review and meta-analysis concluded that there is generally a small risk of congenital malformations and argued against a substantial teratogenic effect of SSRIs [16].

Concerning antipsychotics, data seems to be less abundant and prevents form assessing correctly the teratogenicity of these drugs [14]. Some studies show that second-generation drugs are not associated with congenital birth defects or neurodevelopmental problems [17]. A literature review conclude that antipsychotic drugs do not seem to increase the rate of major congenital anomalies or other foetal problems but studies did not fully consider the possible effects of maternal mental illness [18]. In fact, a study identified that women with severe mental illness had elevated rates of gestational hypertension, gestational diabetes mellitus, smoking and obesity in pregnancy, therefore studies that examine associated risks for severe mental disorders or their treatment should take into account these cofounding co-morbidities and exposures [19]. However, an epidemiologic study put in evidence an increased number of visits to the general practitioner for babies born to mothers on antipsychotics during pregnancy, but this may be biased due to the psychological status of the mothers [17]. The rate of birth defects when their mother has been exposed to antipsychotics is 4 % with clozapine and olanzapine, appearing to be riskier than other antipsychotics [14]. Nevertheless, evidence from a large study suggests that use of antipsychotics in the first trimester of pregnancy generally does not meaningfully increase the risk for congenital or cardiac malformations [20]. A systemic review found malformation rates of 3.5 % for olanzapine, 3.6 % for quetiapine and 5.1 % for risperidone, which does not increase the risk of malformation in a clinically meaningful way [21]. A literature review suggests that there is no increased risk of congenital malformations with second generation antipsychotics but apparent data on other pregnancy outcomes (preterm birth,

Table 2
Malformations encountered in our cohort and corresponding drug exposure.

Malformations	Drugs exposure during pregnancy	Described in the literature (yes/no) (broad PubMed search)
Cornelia de Lange syndrome (1 case)	- Patient 1 : amitryptiline	- No
Syndactyly (1 case)	- Patient 2 : budesonide	- No
Heart murmur (4 cases)	- Patient 3 : trovaflocaxin	- No
	- Patient 4 : norfloxacin	- No
	- Patient 5 : norfloxacin	- No
	- Patient 6 : lamotrigine, levothyroxine, salmeterol, salbutamol	- No for all drugs
Hypospadias (5 cases)	- Patient 7 : phentermine, flufenamic acid, diclofenac, tizanidine	- Yes for all drugs
	- Patient 8 : venlafaxine, lorazepam	- Yes, No
	- Patient 9 : mebeverine	- No
	- Patient 10 : venlafaxine, lorazepam	- Yes, No
	- Patient 11 : doxycycline	- No
Chiari malformation (1 case)	- Patient 12 : fluoxetine	- Yes
Clubfoot (3 cases)	- Patient 13 : hydrochlorothiazide, irbesartan, orlistat	- No for all drugs
	- Patient 14 : haloperidol, lorazepam	- No for all drugs
	- Patient 15 : olanzapine	- No
Ectrodactyly (1 case)	- Patient 16 : venlafaxine, metoclopramide, clotiapine, promazine, mefenamic acid	- No for all drugs
Pelvicalyceal dilatation (4 cases)	- Patient 17 : fosfomycin	- No
	- Patient 18 : minoxidil, betamethasone	- No for all drugs
	- Patient 19 : valacyclovir	- No
	- Patient 20 : valerian	- No
Tetralogy of Fallot (1 case)	- Patient 21 : clomipramine, mirtazapine, clorazepate, lorazepam	- No for all drugs
Syndactyly and ectrodactyly (1 case)	- Patient 22: valproate, cyclophosphamide, alprazolam, prednisone, omeprazole, salmeterol, levofloxacin	- No for all drugs
Pulmonary atresia (2 cases)	- Patient 23 : bendroflumethiazide	- No
	- Patient 24 : methoxypsoralen	- No
Cleft lip (1 case)	- Patient 25 : cabergoline, betamethasone	- Yes for all drugs
Hydrocephalus (1 case)	- Patient 26 : topiramate, venlafaxine	- No, Yes
Cleft palate (1 case)	- Patient 27 : amitryptiline	- No
Prader Willi syndrome (1 case)	- Patient 28 : sertraline, olanzapine, zolpidem, lorazepam	- No for all drugs
Frenulum of tongue (1 case)	- Patient 29 : citalopram	- No
Cardiac malformation (6 cases)	- Patient 30 : methylphenidate, methadone, oxazepam, venlafaxine, zolpidem	- Yes, No, No, Yes, No - Yes, No
	- Patient 31 : methylphenidate, methadone	- No for all drugs
	- Patient 32 : piroxicam, betamethasone	- No for all drugs
	- Patient 33 : furosemide, candesartan/hydrochlorothiazide	- No for all drugs
	- Patient 34 : emcitarabine, tenofovir, raltegravir	- No for all drugs
	- Patient 35 : zolpidem, dalteparin	
Hemangioma (1 case)	- Patient 36 : paroxetine, alprazolam, sertraline, lorazepam, pravastatin	- No for all drugs
Polydactyly (1 case)	- Patient 37 : citalopram, alprazolam, quetiapine, domperidone	- No for all drugs
Cystic fibrosis (1 case)	- Patient 38 : zopiclone, citalopram	- No for all drugs
Renal hypoplasia (1 case)	- Patient 39 : botulinum toxin	- No
Intellectual disability (1 case)	- Patient 40 : duloxetine	- Yes
Renal disability (2 cases)	- Patient 41 : citalopram	- No
	- Patient 42 : spironolactone, losartan, hydrochlorothiazide, atenolol, lisinopril, acetylsalicylic acid	- No for all drugs
Foramen ovale (2 cases)	- Patient 43 : sumatriptan	- No
	- Patient 44 : candesartan, metoprolol, lercanidipine, azathioprine, prednisone,	- No for all drugs
Flaps of periocular skin (1 case)	- Patient 45 : ciprofloxacin	- No
Arachnoid cyst (1 case)	- Patient 46 : balsalazide	- No
Diaphragmatic hernia (1 case)	- Patient 47 : lorazepam, buprenorphine	- No for all drugs
Sacro-coccygeal fossa (1 case)	- Patient 48 : sertraline, olanzapine, alprazolam, zolpidem, esomeprazole, sumatriptan, domperidone	- No for all drugs
Brachial plexus (1 case)	- Patient 49 : citalopram	- No

neonatal adaptation, miscarriage . . .) are insufficient to provide confident estimates [22]. Neonatal complications such as withdrawal symptoms, extra-pyramidal symptoms and respiratory problems may occur after the use of first and second generation antipsychotics in pregnant women [14]. The actual choice of drug for the individual pregnant patient must account for factors other than only safety data and take into account individual disease history, characteristics and treatment response, adverse reaction profile and patients preferences [22]. Moreover a systemic review and meta-analysis indicates that there is an increased risk of gestational diabetes mellitus with antipsychotic exposure in pregnant women, who may benefit from close pregnancy monitoring, lifestyle modifications, early testing for diabetes and targeting modifiable risk factors [23].

The other most frequent ATC class in our cohort were anti-infective drugs. Studies show that the prescription rate increases each trimester during pregnancy with the overall prescription of an antibiotic in 20.8 % of pregnancies [24]. In other studies, it has been estimated that one in four women will have an antibiotic prescription during pregnancy and this represents 80 % of prescriptions for a pregnant woman [12]. The most frequent infections that affect pregnant women are urinary tract infections, pyelonephritis, sexually transmitted infections and upper respiratory infections [12] and during pregnancy, untreated sexually transmitted diseases or urinary infections are associated with a higher risk of morbidity, such as low birth weight, premature birth, or spontaneous abortion [12]. However, exposure to antibiotics during pregnancy is associated also with short- or long-term

effects for children such as congenital anomalies, changes in intestinal flora, asthma, atopic dermatitis [12]. For example, the use of antibiotics during pregnancy could lead to childhood obesity, cerebral palsy or epilepsy, atopic dermatitis, or asthma, for example [12]. However, a recent retrospective study suggests that antibiotic use does not affect the risk of small or low gestational age birth weight or gestational diabetes mellitus in pregnant women [25]. Trimethoprim is associated with an increased risk of cardiovascular malformations or cleft lip and tetracyclines are associated with decreased bone growth and tooth discoloration [24]. However, a systematic review and meta-analysis suggests that the use of quinolone during the first trimester of pregnancy was not associated with an increased risk of birth defects, stillbirths, preterm birth or low birth weight [26]. Nevertheless, in a case-control study and after adjustment for potential cofounders, use of macrolides (excluding erythromycin), quinolones, tetracyclines, sulphonamides and metronidazole during early pregnancy was associated with an increased risk of spontaneous abortion [27].

Our study shows that concerns about drug exposures during pregnancy are more frequent in the first trimester. This can be explained by the fact that the pregnancy is often unplanned and unknown in the first weeks of pregnancy. A counselling on the risk of birth defects is requested as soon as the pregnancy is discovered. For women, treated for a chronic disease, counselling should ideally before pregnancy.

Prematurity is a major determinant of neonatal mortality/morbidity because it has long-term health consequences and premature infants are at increased risk of developing cerebral palsy, sensory and learning disabilities or respiratory problems [28]. Regarding neonatal deaths who are not due to congenital malformations, 28 % are due to preterm birth [28]. The WHO estimated the overall incidence of preterm birth at 9.6 % in 2005 in a systematic review, while the incidence is 7.4 % in Europe and North America [28]. This rate is similar to the rate found in our study suggesting that drug exposure does not increase the risk of prematurity.

The frequency of elective induced abortions is an important indicator of public health, with low rates generally associated with good access to high quality care and good use of contraceptive methods [29]. The elective induced abortion rate in Europe is 10.0 per 1000 women in 2008 [29]. In Switzerland, in the canton of Vaud, between 1990 and 1999, the rate of elective induced abortion was 8.9 per 1000 women with 63 % who declared that they had no contraception [30]. Lately, the Swiss Federal Statistical Office published data on the elective induced abortions in Switzerland and showed that the rate in the canton of Geneva in 2018 is of 12 per 1000 woman of reproductive age (15–44 years old) [31]. These rates are much lower than the one found in our study which is of 10.8 %. This can be explained by several hypotheses. First, most of the drugs reported are those of the central nervous system and may be taken therefore by patients with difficult socioeconomic conditions [30]. In addition, when our counselling service receives the request from the medical doctor, we know that sometimes the patient has already taken the decision to terminate her pregnancy because she considers that drug exposure is too risky or for other personal reasons, even before our teratology counselling. Drug exposure appears to be a risk factor for elective induced abortion, with studies citing a 16 % rate [2] even though drug exposure does not require such an intervention in most cases.

In contrast to this, the 4.6 % malformation rate in our cohort is in the normal range for the general population. Drug exposure does not appear to be a risk factor for malformation in Europe [10].

Spontaneous abortion affects 10–15 % of clinically attended pregnancies and has been linked to both the use of antidepressants and to depression [32]. The rate of spontaneous abortion in our study is lower than this, being of 8 % in this cohort highly exposed to antidepressants/antipsychotics

IUGR is found in 10 % of pregnancies and is associated with higher neonatal mortality/morbidity including prematurity, cerebral palsy, intrauterine death, neonatal death, obesity, hypertension or type II diabetes mellitus [33]. Incidence increases with maternal factors (weight, tobacco, socio-economic status, age, history, pre-eclampsia, anemia . . . etc), foetal factors (multiple gestation, infection, genetic syndrome) or adnexales factors [33]. In our study, IUGR appears in 2.6 % of pregnancies. Withdrawal syndromes and respiratory distress syndromes may be the consequence of the use of central nervous system drugs [33]. These appeared to be low in our study, being of approximately 2 %.

Our study describes the repercussion of drug exposure during pregnancy through our counselling service and has limitations. First, it was sometimes difficult to understand if the termination of pregnancy were voluntary or medically indicated. We also had scarce data on the use of tobacco, alcohol or prenatal vitamins or on the genetic history of parents. Very often, no information was available on the end date of the treatment. Analysis was also done for each drug ken separately and not taking into account polypharmacy. Finally, the outcome of pregnancy is known at the time of delivery only. Malformation, complications or developmental issues occurring later in the child's life were not available.

5 Conclusions

Pregnant women counselled by our information service take between one to seventeen different medications. Drugs are from FDA Pregnancy Category C in 42.9 % of the cases and from the ATC N drug category (nervous system) in 36.4 % of cases. Almost all exposures begin in the first trimester probably because women are not yet aware of their pregnancy. Despite these pregnancies that are all drug-exposed, the rate of malformation at birth of our cohort was of 4.5 %, close to the estimated spontaneous rate of malformation. The rate of the different complications is also close to the rates in the general population.

These data are reassuring about the effects of drug exposure in pregnancy but take into account only the outcome at birth and give no information on long-term developmental issues after drug exposure during pregnancy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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