Various approaches to postmarketing drug safety monitoring

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Abbreviations

ADR	adverse drug reactions
AP	alkaline phosphatase
BP	bisphosphonate
COVID-19	Coronavirus disease-19
CVST	cerebral venous sinus thrombosis
CYP	cytochrome P450
DAA	direct-acting antiviral
DDI	drug-drug interaction
DILI	drug-induced liver injury
DPA	disproportionality analysis
EHRs	electronic health records
EMA	European Medicine Agency
FDA	Food and Drug Administration
ICSR	individual case safety reports
NLP	natural language processing
NPAE	neuropsychiatric adverse events
NSAIDs	non-steroidal anti-inflammatory drugs
PBPK	physiological-based pharmacokinetic
P-gp	P-glycoprotein
RRR	relative reporting ratio
UK	United Kingdom
UMC	Uppsala monitoring center
US	United States
WHO	World Health Organization

Summary

A drug used for its therapeutic effect can also produce adverse effects. Drug safety relates to the potential for adverse effects related to the administration of drugs. The safety profile of marketed drugs is studied during their development. For example, the incidence of common adverse drug reactions (ADR) is estimated during phase III studies. Before being launched on the market, a medicinal product must have been demonstrated of good quality, effective and safe. However, rare ADRs or those occurring after long-term use may not always be detected during the clinical development of a drug. Therefore, continuous drug safety monitoring, or pharmacovigilance, is essential during the whole lifecycle of a drug, including after the market authorization. This allows identifying new risks.

This thesis provides an overview of different tools available for the postmarketing surveillance of drug safety. Classic pharmacovigilance is based on spontaneous reports of individual cases. These cases are typically reported by healthcare professionals to national pharmacovigilance centers and collected in large interactional databases. Some cases of particular interest can be published as case reports or case series. Individual case safety reports have been the basis for many drug withdrawals due to safety concerns, or they can lead to changes in the drug labeling as illustrated by our published case series on atypical femur fractures occurring after long-term use of bisphosphonates. Postmarketing studies are also useful to evaluate safety in certain populations or to assess the impact of certain risk factors. We illustrated the value of such studies by an observational study aiming to describe the safety profile of oseltamivir in children, as well as the impact of some genetic polymorphism on neuropsychiatric adverse events. Besides, drug-drug interactions (DDI) can be a source of ADR when the concentrations of the victim drug are increased by a perpetrator drug. On the other hand, DDI can lead to therapeutic failure when the systemic exposure of the victim drug is decreased by a perpetrator drug, which is also not desirable. We reviewed the potential of DDI between direct-acting antivirals used to treat hepatitis C and opioids, and substances of abuse. Finally, data and text mining strategies are useful when a large amount of data have to be explored, such as global pharmacovigilance databases, electronic health records, or social media. We presented our study applying natural language processing on discharge letters to detect terms related to ADR.

Part 1

Introduction

1. What is drug safety?

Definitions and key concepts

Any substance that is capable of producing a therapeutic effect can also produce unwanted or adverse effects (1). Drug safety relates to the potential for adverse effects associated with the administration of drugs. Modern pharmacovigilance started in the 1960s with the thalidomide disaster, consisting of major fetal malformations caused by the use of thalidomide during pregnancy (2). Pharmacovigilance is defined by the World Health Organization (WHO) as the science and activities relating to the detection, assessment, understanding, and prevention of adverse effects and other drug-related problems (3). There is a diversity of terms used to describe the harmful effects of medicinal products, for example, adverse events, adverse effects, and adverse drug reactions. A clear definition of the key terms and concepts is therefore necessary (4). An adverse event is any untoward occurrence that may present during treatment with a pharmaceutical product but which does not necessarily have a causal relationship to the treatment (1, 3). The terms “adverse effect” and “adverse reaction” are often interchangeable, except that an adverse effect is seen from the point of view of the drug, whereas an adverse reaction is seen from the point of view of the patient (1). In other words, medicinal products cause adverse effects; patients experience adverse reactions (4). An adverse effect is defined as a potentially harmful effect suspected to be resulting from the use of a medicinal product. Adverse effects are usually detected by laboratory tests or clinical investigations (4, 5). They may not always be associated with a clinically relevant symptom. For example, an elevated INR associated with warfarin treatment does not necessarily lead to noticeable harm to the patient such as observable hemorrhage (4). An adverse drug reaction (ADR) is defined by the WHO as a response to a drug which is noxious and unintended, and which occurs at doses normally used in man for the prophylaxis, diagnosis, or therapy of disease, or for the modification of physiological function (3). Another definition of ADR has been given by Aronson to specify the clinical aspects of this term: “an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, usually predicting hazard from future administration and warranting prevention, or specific treatment, or alteration of the dosage regimen, or withdrawal of the product” (5).

One has to distinguish between seriousness and severity. ADRs are considered as serious if they result in death, are life-threatening, lead to, or prolong, hospitalization, involve a persistent disability or incapacity, or are otherwise considered serious by the reporter (4, 6). Severity is a measure of the intensity of the ADR (4). The grading “mild, “moderate” and “severe” is often

used (1). For example, the LiverTox website, which provides up-to-date, comprehensive clinical information on drug-induced liver injury, has developed a 5 point scale for grading the severity of drug-induced liver injury (DILI) (Table 1) (7).

Table 1: Severity grading of drug-induced liver injury (adapted from (7))

Grade	Features
1, mild	Raised serum aminotransferase or alkaline phosphatase (AP) levels or both, but total serum bilirubin <2.5 mg/dL and no coagulopathy
2, moderate	Raised serum aminotransferase or AP levels or both AND total serum bilirubin level ≥2.5 mg/dL or coagulopathy (INR ≥1.5) without hyperbilirubinemia
3, moderate to severe	Grade 2 AND hospitalization because of the DILI
4, severe	Grade 2 AND at least one of the following: • Prolonged jaundice and symptoms beyond 3 months • Signs of hepatic decompensation (INR ≥1.5, ascites, encephalopathy) • Other organ failure believed to be related to DILI
5, fatal	Death or liver transplantation for DILI

When a patient displays an adverse event, the diagnosis of an ADR has to be considered as a differential diagnosis. For example in the case of hepatitis, DILI has to be considered among other causes of hepatitis such as viral hepatitis. Based on several criteria such as the exclusion of other causes and the temporal relationship, we can assess whether the ADR could be due to a drug (causality) (1). One of the most used tools for the assessment of causality is the system developed by the WHO Uppsala monitoring center (UMC), which is an independent center for drug safety and scientific research (Table 2).

Table 2: WHO-UMC system for standardized case causality assessment (adapted from (1, 8))

Causality term	Criteria
Certain	• Clinical event or laboratory test abnormality occurring with a reasonable time relationship to drug administration • Not explainable by concurrent disease or other drugs • Clinically plausible response to the withdrawal of the drug (dechallenge) • Reoccurrence after rechallenge (usually accidental, or if not, only if ethically feasible)

Probable/likely	• Clinical event or laboratory test abnormality occurring with a reasonable time relationship to drug administration • Unlikely explainable by concurrent disease or other drugs • Clinically plausible response to the withdrawal of the drug (dechallenge) • No rechallenge
Possible	• Clinical event or laboratory test abnormality occurring with a reasonable time relationship to drug administration • Explainable by concurrent disease or other drugs • Unclear or no information after drug withdrawal
Unlikely	• The time relationship to drug administration makes a causal relation improbable • Disease or other drugs are plausible explanations
Conditional/unclassified	More data are essential for a proper assessment or additional data are being examined
Unassessable/unclassifiable	Information is insufficient or contradictory and cannot be supplemented or verified

Epidemiology

Adverse drug reactions represent an important cause of hospital admissions, approximately 5-10%, or of excess length of stay in hospitalized patients. The results from different studies cannot always be compared as case definition may differ from one study to another. In a United States (US) prospective study, Bates and al found an incidence of adverse drug events of 6.1 per 100 admissions, defined in their study as an injury resulting from medical intervention related to a drug, which includes overdose for example (the term ADR is restricted to “doses normally used in man”). Analgesics and antibiotics were the most frequently implicated therapeutic classes (9). In a prospective study conducted in the United Kingdom (UK) that included only ADR (i.e. overdoses not included), 6.5% of admissions were judged as being due to an ADR. Non-steroidal anti-inflammatory drugs (NSAIDs) and diuretics were the most frequently implicated therapeutic classes (10). In a French prospective study, Pouyanne et al estimated that 3.2% of hospital admitted patients were admitted because of an ADR. Cardiac, antineoplastic, antithrombotic, and antihypertensive drugs were the most commonly implicated

therapeutic classes (11). In a Swiss prospective study, Wasserfallen et al evaluated that 7.2% of admission to a medical emergency department was caused by an ADR. The most common implicated drugs were as in previously cited studies cytostatic, analgesics, and anticoagulants (12).

Regarding hospitalized patients, several studies and meta-analyses have been published. A Swiss study by Fattinger et al estimated that clinically relevant ADRs occurred in 11% of all hospitalizations. Again, cancer chemotherapeutics were most frequent (13). A study performed in UK hospitals showed that 14.7% of hospitalized patients experienced at least one ADR and that the most commonly implicated drugs were diuretics, opioids, and anticoagulants (14). Lazarou et al performed a meta-analysis of the prospective studies evaluating the incidence of ADRs in hospitalized patients. They found that serious ADRs occurred in 6.7% of US hospitalized patients (15). Finally, a more recent meta-analysis found a pooled ADR cumulative incidence of 16.88% (16). A part of occurring ADR may be preventable. In a meta-analysis conducted in outpatients and inpatients, about half of ADRs were preventable (17).

These studies assessing the epidemiology of ADRs are based on the known safety profile of marketed drugs. The safety profile is documented throughout the whole drug development, from in vitro studies, studies in animals, and finally clinical studies.

2. Drug clinical development

After in vitro, eventually, ex vivo and/or in silico studies, and animal testing, new drugs have to be evaluated through clinical trials before approval. These preapproval clinical trials are typically classified into three phases, from phase I to phase III, while Phase IV studies are conducted once the drug has been approved (18). Phase I studies allow establishing the pharmacokinetic, pharmacodynamic, safety, and tolerability profile of a novel drug candidate. They are usually conducted in a limited number of healthy volunteers (20 to 80), except with oncology drugs because of evident ethical issues (18, 19). Phase II studies enroll participants who have the disease of interest, therefore, aiming to test the clinical effectiveness and the safety of the novel drug at a therapeutic dose in the intended patient population (18-20). They typically include several hundred participants (18). Phase III studies, often referred to as pivotal trials, aim to demonstrate the efficacy in a large population with the disease or clinical condition for which the drug is being developed and to estimate the incidence of common adverse reactions (18, 20). They often should include certain subgroups of participants that are representative of patients who will receive them in clinical practice once the drug is approved,

for example, elderly patients (18). Phase III studies are usually randomized controlled trials conducted in several thousands of participants (18, 19).

If the investigational drug has been demonstrated safe and efficacious in the intended population, the sponsor may submit a marketing authorization application to drug regulatory authorities (e.g. the European Medicine Agency – EMA, the Swiss Agency for Therapeutic Products – Swissmedic, the Food and Drug Administration – FDA). After a thorough scientific evaluation, the drug authorities approve or not the drug (20, 21). Sufficient evidence is required to show the new drug to be of good quality, effective and safe. The criteria of quality and efficacy must be met, whereas the issue of safety is less certain (3). Safety is not absolute, but at the time of authorization, marketed medicinal products are judged as having a positive benefit-risk balance.

3. The importance of postmarketing pharmacovigilance

Given the limited sample size of preapproval studies, very rare ADRs have a low probability of being detected. Phase III studies are usually designed to demonstrate efficacy endpoints. They don't have the statistical power to establish an adverse event rate of less than 1 in 100 persons, which underscores the importance of postmarketing surveillance in identifying less-common adverse drug reactions (ADR) (20). It is estimated that the sample size that would be needed to identify a single case of an adverse event of interest is approximately 3 times the reciprocal of the frequency of this event in the general population. For example, if an event spontaneously occurs in 1/10000 individuals (background rate), a large sample size of 30000 participants is needed to observe at least one event with 95% confidence. In practice, such large studies are infeasible from both cost and time perspectives (18). Therefore, continuous and careful monitoring of the safety profile of all medicines throughout their lifecycle, including during the postmarketing phase, is essential in identifying and minimizing new risks (22).

4. Different tools for the postmarketing surveillance of drug safety

Pharmacovigilance traditionally emerged with spontaneous reporting (23). Nowadays modern pharmacovigilance needs a comprehensive approach looking at ADR-related information arising from complementary data sources (
Figure 1) (24).

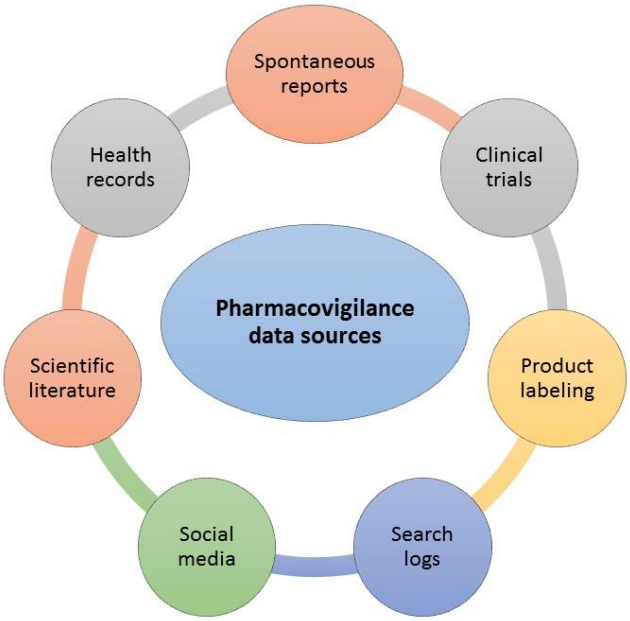

Figure 1: Different data sources used for pharmacovigilance (adapted from (24))

Individual case safety reports

Individual case safety reports (ICSR) describe what happened to a patient after taking a drug. Such cases emerge from a reporter (usually the prescriber or other care professionals, eventually the patient) and describe a patient, a drug, and an event (25, 26). There are legal obligations on pharmaceutical companies to create an ISCR from each adverse event associated with any of their marketed products and to submit them to drug regulatory authorities (26). In Switzerland, under the Therapeutic Products Act, healthcare professionals (medical doctors, pharmacists, etc.) are obliged to report suspected ADRs to the Swissmedic National Pharmacovigilance Center. All serious adverse reactions that are hitherto unknown or insufficiently documented in the product information leaflet of the medicine concerned, as

well as any other medically significant adverse reaction, must be reported (6). The whole system however suffers from under-reporting. It is estimated that approximately 5 to 10% of ADRs are effectively reported by healthcare professionals (27). Cited reasons for under-reporting include poor knowledge of how to use the spontaneous reporting systems, conflicts of interest, forgetfulness, lack of time, and uncertainty about causal relationships between drugs and adverse events (28). ICSR submitted to national pharmacovigilance centers are then collected by the WHO international drug monitoring program (or UMC – Uppsala Monitoring Center), in a global pharmacovigilance database, the WHO Vigibase (26). Vigibase contains, as of June 2019, more than 20 million ADR reports. Currently, 139 countries have a full membership to the WHO Program for International Drug Monitoring, and 32 are associate members (27). Two other major global pharmacovigilance databases have requirements for international reporting of individual case harm reports: the US FDA AERS (Adverse Event Reporting System) database and the EU Eudravigilance database. There are some differences between the data in these databases, but also many overlaps and duplications (29). The Eudravigilance database is operated by the EMA (30) and contains over 16 million ICSR (27). Single cases by themselves cannot generally provide enough information to make regulatory decisions. However, the existence of multiple similar cases can generate an alert (a signal) that will justify further exploration (25). Therefore, all these global pharmacovigilance databases can be used to detect a signal, defined as “information arising from one or multiple sources, including observations and experiments, which suggest a new potentially causal association, or a new aspect of a known association between an intervention and an event or set of related events, either adverse or beneficial, that is judged to be of sufficient likelihood to justify verificatory action” (22).

In some cases, the identification of new risks/new ADRs identified through spontaneous or case reports during the postmarketing phase can lead to the market withdrawal of the drug (27). Onakpoya et al reviewed the postmarketing withdrawal of 462 drugs due to ADRs that occurred between 1953 and 2013. The withdrawal was based on case reports in 71% of the cases. Randomized and non-randomized studies represented respectively 5.8 and 9.3% of the evidence leading to withdrawal. The most commonly reported types of ADRs leading to withdrawal were hepatotoxicity, immune-related reactions, cardiotoxicity, and neurotoxicity (approximately 15% each) (28). A similar study was performed on 21 drugs withdrawn in France between 1998 and 2004. As in Onakpoya’s publication, evidence leading to drug withdrawal came from spontaneous case reports (or case series) in the vast majority of the cases (19 out of 21). Similarly, the most frequent ADRs were hepatic (n=7), cardiovascular (n=4) and neurological (n=3) (31).

Case reports and case series are usually targets of criticism (32) because they are traditionally viewed as low in the evidence hierarchy. However, they remain precious tools for identifying

new, previously undescribed associations between drug exposures and ADRs. The careful description of well-documented spontaneous case reports can provide enough evidence for drug authorities to take regulatory measures including changes in labeling or drug withdrawal from the market (31). As an example, our description of eight patients presenting with atypical low-energy femur fracture after long-term treatment with bisphosphonates led to a change of the drug labeling in Switzerland – ***see full article in Part 2*** (33). A review of 130 publications (case reports or case series) showed that among 9 criteria defined as important to report (e.g. demographic characteristics, exposure duration, doses, time from initiation to the ADR, clinical and laboratory, etc.), an average number de 6 criteria was reported. Co-morbidity was the criterion that was most often missing (34).

In conclusion, case reports and case series are still the cornerstones of pharmacovigilance and the basis of the majority of drug market withdrawals (31, 34).

Postmarketing studies

Postmarketing studies are usually named phase IV studies. Although in the past, phase IV studies were synonymous with postmarketing surveillance or pharmacovigilance, not all postmarketing studies are limited to safety issues. Phase IV studies may include drug-drug interaction studies, studies in special populations (e.g. pediatric patients, elderly patients), equivalence testing, or pharmacoeconomic studies (35). Those studies are not always large studies designed to detect very rare ADRs. Zhang et al recently performed an overview of 4722 phase IV studies registered in ClinicalTrials.gov and focusing on safety or safety/efficacy endpoints. Among the included studies, 330 focused on safety alone, and 4392 on safety/efficacy. The median number of participants per trial was 104.0 (interquartile range: 48.0–258.0). Of the total phase IV trials evaluating drug safety alone, 68.5% had an enrolment of fewer than 300 patients, and only 3.9% (n=13) of them enrolled more than 3000. Of the phase IV trials evaluating drug safety alone, 21.8% enrolled children (36).

The specific case of pediatric drug safety

During drug development and the postmarketing surveillance phase, the quantity of information on adults grows as the medicinal product is marketed and used in large populations of adults. But in children, the situation is different. The pediatric population is often excluded from trials conducted during the clinical development of drug candidates. Therefore limited information on dose recommendations, efficacy, and risks in children is available, making off-label use frequent (37). It is estimated that off-label use concerns over 50% of prescribed drugs in children (38). As for adults, spontaneous reports for children are important. For rare reactions, published literature cases might be the only source of ADR information for clinicians

(37). According to a recent Swiss publication, only 8% of all ICSR reported in Vigibase in 2018 involved children, whereas the pediatric population represents 20% to 30% of the whole population in developed countries, reaching 50% in some African countries (38). Hypotheses that could explain the weak reporting rates in children include lower drug prescription and difficulties to recognize ADR particularly in small children who have limited ways to communicate discomfort (37, 38).

Other strategies can be applied to promote pharmacovigilance in pediatrics, for example, active surveillance strategies. Active surveillance systems can optimize the detection of ADRs when supported by standardized methodology. Such strategies can serve important purposes such as exploring specific drug safety concerns or be responsive to drug safety or public health emergency situations (e.g. intensive safety surveillance of antiviral drugs during an influenza outbreak) (39). As an example of active surveillance strategy performed through a phase IV study, we performed a cohort study in the pediatric population during the H1N1/09 influenza pandemic to actively assess the safety profile of oseltamivir in children – ***see full article in Part 3*** (40).

The specific role of pharmacogenetics in drug safety

Although many factors influence the effect of drugs (e.g., age, organ function, drug interactions), genetic factors account for a significant proportion of drug response variability, including the occurrence of ADR (39). This applies to adults but also children. Although certain genetic polymorphisms have been recognized as risk factors for ADR in children, several issues have restrained investigation in pharmacogenomics in children, which historically included the amount of blood needed and ethical considerations (41).

From a general point of view, genetic polymorphisms that can affect drug response can concern enzymes, transporters implied in drug pharmacokinetics, or molecules (e.g. receptor) implied in drug pharmacodynamics (42). Pharmacogenetic tests can be used virtually during any stage of drug development. For example, during phase III, these tests have the potential to exclude subjects at high for ADR by genotyping (e.g. HLA-B*57:01 as a strong risk factor for abacavir-related hypersensitivity reaction). In phase IV studies or the context of pharmacovigilance, they can help to understand for each ADR whether or not the ADR was due to a genetic variation, with a potential for label changes and significant risk reduction in the future. Pharmacogenetic studies in cohorts in their natural settings, in the absence of controls, is an example of such phase IV studies. They consist to observe and follow one study sample receiving a therapy, and analyzing the effects of genotypes on the study outcome. As an example of such study conducted in a real-world setting, our previously mentioned pediatric study also evaluated the impact of *ABCB1* genetic polymorphisms on oseltamivir-induced neuropsychiatric adverse events – ***see full article in Part 3*** (40).

Such design reflects true medical reality and is easy to perform but may display a significant risk of confounding (43).

Role of drug-drug interaction in drug safety

A drug-drug interaction (DDI) is defined as a change in the effect of a drug as a result of the interaction with one or more drugs. DDIs are generally classified as pharmacokinetic or pharmacodynamic. They may result in a reduction (e.g. therapeutic failure) or an increase in therapeutic effects, including ADR (44). More than 20% of the reported adverse reactions are associated with a DDI (45). In a recent systematic review and meta-analysis, the pooled prevalence of clinical relevant DDI in hospitalized patients was 9.2%, ranging from 1.2% to 64.0% in individual studies (44). In an earlier conducted meta-analysis focusing on hospital admissions, the median prevalence rate of hospital admissions associated with DDIs in the total population was 1.1%. When restricting the analysis to patients admitted for ADR, the median prevalence rate associated with DDIs in ADR patients was 22.2% (46).

Two recent studies on DDI and ADR were specifically conducted on pharmacovigilance databases. In the French database, between 2012 and 2016, 2% (=4027) of the 190261 reported ADR cases were identified as resulting from a DDI. Most of them (82%, 3303) were considered serious and 274 were fatal (7%). The most frequently incriminated drugs in serious DDI cases were fluindione (11%), aspirin (8%), clopidogrel (3%), warfarin (3%), and amiodarone (3%). Not surprisingly given the implicated drugs, the most commonly reported ADR was hemorrhage (40%), followed by renal failure, pharmacokinetic alteration, and cardiac arrhythmias (approximately 5%) (47). In an Italian database from the Veneto Region, between January 2015 and May 2020, 2195 serious reports containing at least two drugs were reviewed. Among them, 381 reports (17.4%) described an occurring ADR associated with a DDI. As in the French database, vitamin K antagonists (warfarin) were the most frequently reported interacting drugs and the most common ADRs were gastrointestinal or cerebral hemorrhagic events (48). To illustrate the importance of DDIs in drug safety and also drug efficacy, we performed a literature review of DDIs between direct-acting antiviral (DAA) drugs and potentially concomitantly used substances, such as opioids, stimulants, and alcohol, suggesting limited DDI potential – ***see full article in Part 4*** (49). For example, methadone, used in opioid substitution therapy, has been associated with QT interval prolongation. A drug increasing its systemic concentrations may increase the risk of prolonged QT and of “torsade de pointes”. Such interaction is not expected with DAAs.

Finally, one has to take into account the role of genetic variation in the potential for developing ADR following a DDI (drug-drug-gene interactions). Two possibilities of drug-drug-gene

interactions exist. The genetic variant and the perpetrator drug combine to act on transporter or metabolism pathways to greatly alter drug concentrations. Conversely, we can observe phenoconversion, when the interacting drug effect and the genotype have opposing effects, resulting in a temporary phenotype shift e.g. neutralizing/reversing the effect of a gain of function genotype when an inhibitory drug is prescribed (50).

The value of data and text mining in drug safety

Data mining is the process of seeking interesting or valuable information within large data sets, such as large spontaneous reports databases or in electronic health records (EHRs). In pharmacovigilance, data mining has the potential to discover complex interactions that defy human recognition. In spontaneous reports databases, the majority of ICSR represent “noise” because the reported ADR is already labeled or non-serious. Applying statistical approaches allows identifying new possible ADRs (signals) (51). One of them is disproportionality analysis (DPA). The basic concept of DPA is to quantify the degree to which a drug-event combination occurs “disproportionally” as compared with what would be expected if there were no statistical association between the drug and the event. The WHO uses a Bayesian version of the relative reporting ratio (RRR) to monitor signals in Vigibase. The RRR is defined as the ratio of the observed incidence rate of a drug-event combination to its “baseline” expected rate under the assumption that the drug and event occur independently (52). In other words, the proportion of an event reported with a drug among all events reported for the drug is compared to the same proportion for a comparator population (25). A value of close to 1 for any of these measures supports the hypothesis that there is no association between the drug and the event. An RRR value of 3, for example, would indicate that there are three times as many drug-event reports in the database as would be expected, which might support the hypothesis of a drug-event association (52).

Limited information is available in spontaneous reports. Therefore EHRs represent a complementary source of data for pharmacovigilance with the availability of more comprehensive medical information obtained during the course of clinical care (53). EHRs contain the patient medical information such as diagnosis, prescriptions, results of lab tests and other exams, results of specialist visits, or hospital discharge summaries (25). From a pharmacovigilance point of view, they contain elements of interest such as the timing of medication administration, symptom development, and detailed clinical history (53). For pharmacovigilance purposes, EHRs can be subject to expert manual chart reviews (25), which can be expensive in terms of manpower and time (53). Computerized monitoring systems implemented in EHR can help the automated detection of ADRs. These systems can scan for

trigger words in free text (e.g. “toxicity”, Lyell’s syndrome” etc.) or they can be based on signals generated by structured data such as laboratory or prescription systems (54). Text mining is defined as the process of extracting meaningful information from large amounts of unstructured text using computational methods. In its most sophisticated approach, it is based on statistical, machine learning, and linguistic techniques that are associated with natural language processing (NLP). For pharmacovigilance purposes, NLP typically uses terminologies from various dictionaries such as Anatomical Therapeutic Chemical classification system (ATC) (coding for drugs), International Statistical Classification of Diseases (ICD) (for diseases), Medical Dictionary for Regulatory Activities (MedDRA) (includes terms for symptoms, diseases, indication) and Systemized Nomenclature of Medicine Clinical Terms (SNOMED CT) (multilingual medical terminology that includes diagnoses, procedures, anatomy) (24). Different NLP methods exist for EHR-based pharmacovigilance. The keyword and trigger phrase search relies on keyword and trigger phrase matching in narratives. These triggers can correspond to medications, diseases, or symptoms. For example, the word “stopped” can be suggestive of a drug withdrawal that may be due to an ADR. The word “naloxone” is suggestive of opioid-related ADR or overdose. Symbolic methods extend keyword and trigger phrase searches by exploring the syntactic and semantic patterns surrounding the drugs and adverse events. They include using rules, regular expressions, and syntactic grammar to specify the patterns for the presence of an ADR (55). As an example, we performed a study on discharge letters evaluated the potential of NLP to identify ADR in comparison to expert manual annotation based on defined rules – ***see full article in Part 5*** (56). According to a review of 7 studies, the performances of NLP techniques considerably varied across studies, and many ADRs were missed compared to manual chart review. However, NLP can be a valuable supplement, in particular when screening large amounts of text (57). Challenges remain to properly identify signals that are more relevant and credible with respect to ADRs (55).

Regarding mining of structured data, recently Ramirez et al evaluated the incidence of suspected serious ADRs in Coronavirus disease-19 (COVID-19) patients as detected by a pharmacovigilance program by laboratory signals implemented in the hospital’s EHR. Detected cases were manually reviewed. They found that serious ARDs were suspected in 35% of COVID-19 patients detected by their program based on laboratory levels, with DILI being the most frequent ADR. The drugs most frequently associated with serious ADRs were tocilizumab, dexketoprofen, azithromycin, lopinavir-ritonavir, dexamethasone, and chloroquine/hydroxychloroquine (58).

Finally, data mining techniques can be applied to social media, which have million to billion active users, for pharmacovigilance purposes. The quantity and near-instantaneous nature of social media provide potential opportunities for real-time monitoring of ADRs. However, social media as a source of ADRs pose some challenges such as the variety of ways in which drug

names (brand name, active ingredients) and reaction terms (not always found in medical lexicons) can be described (59). As an example, in a study evaluating terms posted on Twitter and Facebook, millions of events were discussed among which the most commonly occurring terms were pain, altered state of consciousness, headache, malaise, and drug ineffective. The most frequently discussed drugs were diphenhydramine, influenza vaccine, dextroamphetamine, codeine, and morphine. The percent of proto-adverse events, defined as the posts in which a potential event is discussed within the context of drug use varied from 3 to 51% (60). According to a recent systematic review that included 38 studies using social media, the information in identified serious and unexpected proto-adverse events was of poor quality and rarely allowed the evaluation of causal relationships. Therefore social media cannot be currently recommended for routine pharmacovigilance. However, they may have the potential to anticipate pre-specified known signals, and the patients' perception as reported in posts can help implement effective risk communication strategies (61).

Part 2

Individual case safety reports for postmarketing surveillance

Introduction to the article

“Ing-Lorenzini K, Desmeules J, Plachta O, Suva D, Dayer P, Peter R. Low-energy femoral fractures associated with the long-term use of bisphosphonates: a case series from a Swiss university hospital. Drug Saf. 2009;32(9):775-85.”

In this article, we describe eight patients admitted to the orthopedic surgery division of our hospital with low-energy femoral fractures after long-term therapy with bisphosphonate (BP) drugs. During the previous years before we were aware of these cases, the medical literature reported several case reports and case series alerting of this potential adverse effect of long-term BP therapy. The description of our cases led to a change in the Swiss label of BPs used in osteoporosis, i.e. alendronic acid and ibrandronic acid. This adverse effect was later considered as a class effect of BP by drug authorities. Although BPs reduce the risk of osteoporotic fractures, they also can cause femoral shaft or subtrochanteric fractures, which are rare and atypical as compared to the traditional localization of osteoporotic fractures (wrist, spine, and hip). These atypical fractures generally occur after minimal trauma and are associated with cortical thickening, which can also be observed in the contralateral femur. In our patients, they occurred after at least one year of BP treatment. BPs act by reducing bone turnover, thereby increasing bone mineral density. This leads to a reduced risk of osteoporotic fractures. However, too highly mineralized bones may become brittle, leading to the development of microdamages. If bone resorption is inhibited as under BP therapy, the physiological process of bone-remodeling repair is reduced and the natural repair of lesions cannot take place, eventually leading to their local extension and fatigue fracture. Our article underscores the importance of postmarketing surveillance and of case series in detecting ADRs that are rare and that develop after long-term use.

SHORT COMMUNICATION

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Low-Energy Femoral Fractures Associated with the Long-Term Use of Bisphosphonates

A Case Series from a Swiss University Hospital

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Abstract

Background: Bisphosphonates are effective and well tolerated anti-resorptive drugs used for the treatment of osteoporosis. However, some concerns about their potential long-term negative effects are emerging.

Objective: We report a series of patients with a history of bisphosphonate treatment admitted to our institution with a low-energy subtrochanteric fracture.

Patients and methods: Eight patients fulfilling these two criteria within the last 2 years were included in our retrospective analysis. All cases were reported to the Swiss National Pharmacovigilance Centre.

Results: All patients presented with a typical radiological pattern consisting of a cortical thickening at the lateral femoral subtrochanteric cortex with a horizontal fracture line originating precisely at this level. Four patients eventually developed a stress fracture or complete fracture of the contralateral femur. Two patients demonstrated delayed healing of their fracture. Five patients had been on alendronate therapy for a period ranging from 16 months to 8 years, two had been on ibandronate for 4 months and 1 year, respectively, after changing from alendronate, and one patient had been on pamidronate until 1 year before the fracture occurred. Seven patients were also receiving long-term proton pump inhibitor (PPI) treatment which could have contributed to the increased risk of fracture. Four patients were receiving both PPI and long-term corticosteroid treatment. The hypothesis of a negative pharmacodynamic interaction between bisphosphonates, PPIs and corticosteroids which could lead to a decrease in bone strength after long-term use needs further investigation.

Conclusion: Prescribers should be aware of the possibility of these rare adverse reactions and the prolonged use of bisphosphonates should be reconsidered until long-term robust safety data are available.

Background

Osteoporosis is a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture. It is in part a natural consequence of aging in postmenopausal women, but can also be drug induced (e.g. by corticosteroids). The most common consequences of osteoporosis are fractures of the hip, wrist and vertebrae.[1] Most currently available osteoporosis drugs are anti-resorptive agents that act to decrease bone turnover, such as the bisphosphonates (e.g. etidronate, alendronate, risedronate).[1-3] Bisphosphonates are synthetic, nonhydrolyzable analogues of naturally occurring pyrophosphates. They inhibit bone resorption through their effects on osteoclast function. These agents demonstrated a clinically important benefit in the secondary prevention of the majority of osteoporotic fractures. However, some concerns about their potential long-term negative effects are emerging.[4,5] Recently there have been several reports in the literature of atypical low-energy fractures occurring in patients who had been treated with alendronate for 1–10 years.[6-14]

An increase in the frequency of subtrochanteric fractures, all sharing the same typical radiological aspect, involving a cortical thickening at the lateral subtrochanteric cortex with a horizontal fracture line originating at this precise level and eventually extending medially, was noticed in our institution. As this fracture pattern is quite uncommon in osteoporotic patients, we retrospectively reviewed these cases to assess the use of bisphosphonates. This is a report of the first eight patients fulfilling these criteria. This report follows the guidelines for submitting adverse event reports for publication recently proposed by a task force composed of members of the International Society for Pharmacoepidemiology (ISPE) and of the International Society of Pharmacovigilance (ISoP).[15]

Patients and Methods

Cases of low-energy or spontaneous femoral fractures presenting with the same typical radiological appearance in patients admitted to our division of orthopaedics and trauma surgery within the last 2 years were analysed. Nine cases were identified. Only patients with a history of bisphosphonate treatment were retained for our case series. Eight patients fulfilled these two criteria (low-energy femur fracture and bisphosphonate treatment). The ninth patient had never received bisphosphonate treatment.

Clinical data and drug history were obtained from the patients' medical records and via telephone interviews with the GP or patient by a staff member of the regional pharmacovigilance centre.

All cases were then reported to the Swiss Health Authorities and consequently to the WHO Collaborating Centre for International Drug Monitoring, the Uppsala Monitoring Centre (UMC), in Uppsala, Sweden.

Results

Case Presentation

The history and clinical findings of these eight patients are described in table I. Relevant co-morbidities and concomitant therapies are described in table II. Their average age at the time of the first fracture was 67.5 years and seven patients (88%) were female. All patients were admitted with a fracture that developed following a fall from standing height or less; the fractures were even spontaneous in some cases.

In addition to biphosphonates, all patients were receiving oral calcium supplementation. Five patients (1, 2, 3, 7 and 8) were being treated with alendronate at the time of fracture. The duration of alendronate therapy in those receiving alendronate at the time of fracture ranged from 16 months to 8 years. Patient 7 had been

Table I. Summary of patient details

Case	Sex	Age (y)[a]	Mechanism	Fracture	Contralateral stress reaction/fracture	Bisphosphonate (duration of treatment before first fracture)	Indication	Pain before	BMD[b]/T-score[c] (date)
1	F	86	Slipped and fell	Right femoral shaft in 2007	No	Alendronate (16 mo)	Osteoporosis	No	0.615/–2.80 (2006)
2	M	61	Spontaneous pain while on the stairs	Right subtrochanteric in 2004	Thickened cortex in 2004, stress fracture in January 2008	Alendronate (2 y)	Osteoporosis	Yes, on the left	NA
3	F	79	Details not available	Left subtrochanteric in 2005	Thickened cortex in 2006, femoral shaft fracture in 2007 after falling from a standing height	Risedronate (duration unknown), then switched to alendronate (2 y)	Osteoporosis	No	0.628/–2.09 (2001)
4	F	57	Fell from a standing height	Right subtrochanteric in February 2006	Thickened cortex in April 2006, femoral shaft fracture in September 2006 after accidentally falling	Alendronate (10 y in total), then switched to ibandronate (4 mo before first facture)	Corticosteroid-induced osteoporosis	No	NA
5	F	67	Fell from a standing height	Right subtrochanteric in 2006	No	Alendronate (3 y in total), then switched to ibandronate (1 y)	Osteoporosis	No	NA
6	F	61	Tripped and fell	Left subtrochanteric in 2007	No	Pamidronate (5 y; stopped 1 y before fracture)	Osteoporosis	No	NA
7	F	66	Fell after pain	Left subtrochanteric in 2003	Thickened cortex, femoral shaft insufficiency fracture diagnosed in January 2008	Alendronate (5 y in total, 2 y before first fracture), then switched to ibandronate (1 y before second fracture)	Osteoporosis	Yes, both sides	0.614/–2.12 (2001)
8	F	63	Slipped and fell	Right subtrochanteric in 2008	No	Alendronate (8 y)	Osteopenia	No	0.657/–1.7 (2004)

a Age at the time of the first fracture.

b Bone mineral density (BMD) of the femoral neck (g/cm^2); most recent value available.

c The T-score is the bone density value expressed in relation to a young healthy population in standard deviation (SD) units. According to the WHO criteria, osteoporosis is defined as a BMD that lies 2.5 SD or more below the average value for young healthy women.[16]

F = female; **M** = male; **NA** = not available.

Table II. Patients' relevant co-morbidities and therapies

Case	Sex	Age (y)	Significant co-morbidities	Corticosteroid treatment	Proton pump inhibitor treatment	Calcium supplementation	Calcium levels[a] (date)
1	F	86	Hypertension, atrial fibrillation, hiatal hernia and gastro-oesophageal reflux	N	Y (pantoprazole)	Y	NA
2	M	61	Rheumatoid arthritis (treated by prednisone and methotrexate), hypertension	Y (oral prednisone)	Y (esomeprazole)	Y	2.15 (2004)
3	F	79	Hypertension, gastro-oesophageal reflux	N	Y (esomeprazole)	Y	NA
4	F	57	Chronic obstructive pulmonary disease (since 1968), dyslipidaemia, hiatal hernia	Y (oral and inhaled corticosteroids)	Y (esomeprazole)	Y	2.03 (2006)
5	F	67	No significant co-morbidities	N	N	Y	NA
6	F	61	Renal transplantation in 1994 (treated by prednisone, azathioprine and ciclosporin), hypertension, reflux, postmenopausal treatment (transdermal estradiol)	Y (oral prednisone)	Y (esomeprazole)	Y	NA
7	F	66	Postmenopausal treatment (tibolone), chronic low back pain, gastro-oesophageal reflux, hypertension, asthma	Y (inhaled corticosteroids)	Y (rabeprazol)	Y	2.34 (2007)
8	F	63	Hypertension, hypercholesterolaemia, hypothyroidism	N	Y (esomeprazole)	Y	2.42 (2008)

a Units: mmol/L; normal values: 2.20–2.52; most recent value available.

F = female; **M** = male; **N** = no; **NA** = not available; **Y** = yes.

receiving alendronate for 2 years at the time of the first fracture. She continued this treatment for 3 additional years and then changed to ibandronate for 1 year before an insufficiency fracture occurred on the contralateral femur. Two patients (4 and 5) were being treated with ibandronate at the time of their first fracture, but had previously been treated with alendronate for 10 and 3 years, respectively. Finally, patient 6 had only been treated with pamidronate for 5 years, but had ceased treatment 1 year before the fracture occurred. Two patients (6 and 7) were also receiving hormone replacement therapy. Three patients (2, 4 and 6) were being treated with long-term oral corticosteroids and patient 7 was being treated with inhaled corticosteroids. Finally, all patients except one (5) were receiving a proton pump inhibitor (PPI), in most cases as a long-term treatment of gastro-oesophageal reflux.

Bone mineral density (BMD) measurements of the femoral neck and calcium values were available for four of our patients (tables I and II).

Figure 1 shows a representative radiograph of the femoral fractures observed in our patients, generally consisting of cortical thickening and a transverse fracture. Four patients (2, 3, 4 and 7) also developed a stress fracture or complete fracture of the contralateral femur in a period of months to years after the first fracture. Two patients (2 and 3) demonstrated delayed healing of one of the fractures.

A bone biopsy was obtained in two patients (2 and 7). Figure 2 shows a photomicrograph of the biopsy of the cortex obtained in patient 7. This patient had only been treated surgically for a subtrochanteric fracture of the left femur in 2003. In January 2008, she presented to us with complaints of pain in her right thigh and had radiographic findings of cortical thickening in the proximal shaft. She underwent prophylactic intramedullary nailing and biopsy. The biopsy shows that the fracture crosses the whole thickness of the lateral cortex. While partial bone bridging can be seen in the periosteum, suggesting a chronic process, there is a total absence of fracture healing or even remodelling within the cortex.

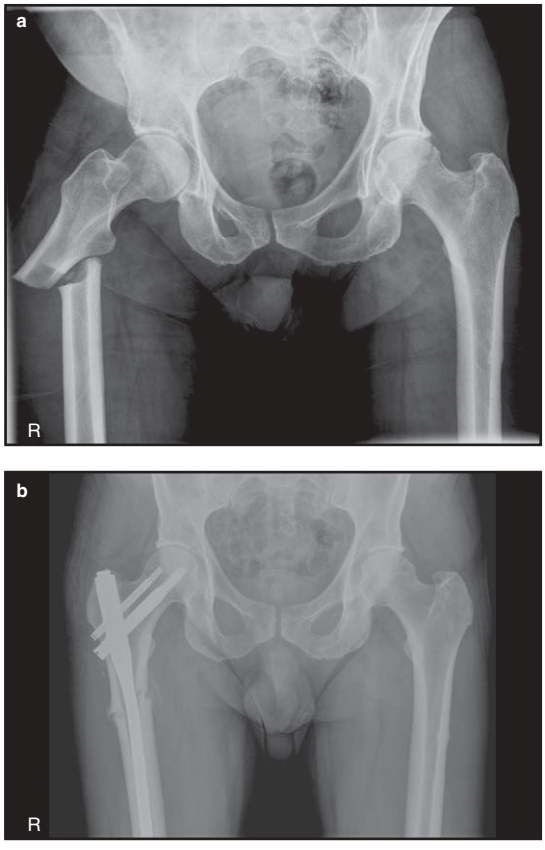

Fig. 1. (**a**) Radiograph of a spontaneous subtrochanteric fracture of the right femur presented by patient 2 in 2004 when he was 61 years of age. At that time, he had been receiving alendronate treatment for more than 2 years. A thickening of the lateral cortex of the femur with a transverse fracture originating at this level, a very typical radiological finding, was observed. A similar cortical thickening was evident on the contralateral femur, although the patient was asymptomatic at that time. (**b**) Radiograph after intramedullary fixation. A delayed union of the fracture was observed 6 months later, but the fracture finally healed after 12 months. This patient was seen again 3 years later with severe left thigh pain. Radiographs were consistent with a stress fracture of the left femur at the level of the cortical thickening. Prophylactic fixation is being considered at this time. **R** = right-hand side.

Outcome and Follow-Up

All eight patients required intramedullary nailing of their fracture. Two patients developed delayed healing of the fracture, and a second surgery due to non-union was required in one of these two patients to obtain union. Four of our eight patients exhibited cortical thickening in the contralateral femur. This cortical thickening was observed either at the time of the initial fracture or several months to years later. Two of these four patients developed a stress fracture, one of which required a prophylactic intramedullary nailing. The two other patients developed a complete fracture and required intramedullary nailing.

Discussion

We describe here eight cases of femoral shaft or subtrochanteric fractures occurring after at least 1 year of bisphosphonate treatment. Insufficiency fractures of the femoral shaft are rare, contrary to the traditional triad of wrist, spine and hip fractures seen as the defining feature of osteoporosis.[6] The proximal femoral shaft and subtrochanteric region is subject to high stress and not expected to fracture from minimal trauma unless there is severe underlying metabolic bone pathology. Neviaser et al.[14] did not observe such a fracture pattern in their untreated patients with osteoporosis, which suggests that this condition alone is not sufficient to cause this specific failure of the femoral shaft.

The clinical benefit of bisphosphonates in the secondary prevention of osteoporotic fractures has been demonstrated by several studies.[1-3] More recently, the efficacy of zoledronic acid once yearly has also been demonstrated.[17] No increased incidence of serious adverse effects were detected with alendronate, etidronate, risedronate or zoledronic acid, but the potential risk of upper gastrointestinal events and, less commonly, osteonecrosis of the jaw and atrial fibrillation could not be ruled out. Participants from FIT (the Fracture Intervention Trial)[18-21] who received alendronate during at least three years were eligible for the FLEX (the FIT Long-term Extension), which lasted 5 years.[22] The authors concluded that the long-term safety of alendronate was confirmed by this study. No increased fracture risk was observed with the long-term use of alendronate. The conclusion of the FLEX study confirmed previously published results from Bone et al.[23]

Nevertheless, several recent publications have reported low-energy fractures associated

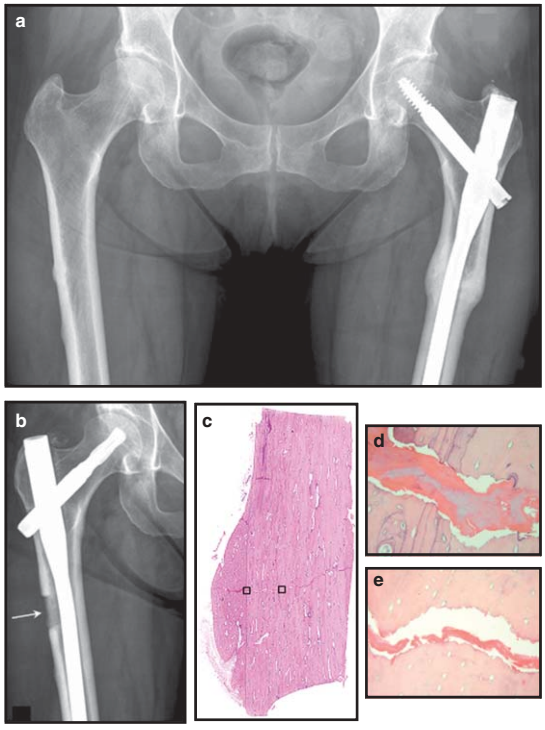

Fig. 2. (**a**) Radiograph of a spontaneous subtrochanteric fracture of the left femur presented by patient 7 in 2003 when she was 66 years of age, after intramedullary fixation. At that time, she had been receiving alendronate treatment for more than 2 years. (**b**) In January 2008, she presented with severe pain in her right thigh. Radiograph showed a subtrochanteric insufficiency fracture line. She underwent prophylactic intramedullary nailing and a biopsy of the lateral subtrochanteric cortex (arrow) at the same time. (**c**) Photomicrograph of the biopsy (haematoxylin and eosin stain). Two representative regions of the biopsy (squares) are shown in (**d**) and (**e**). (**d**, **e**) High magnification (×400) of the left and right squares, respectively. The fracture line is filled with blood, and no cellular reaction or osteoclasts are visible.

with the long-term use of alendronate. Four case reports concerning single patients have been published in 2006 and 2007.[6-9] In 2005, Odvina et al.[10] reported the first case series of nine patients. They described the development of spontaneous non-spinal fractures in patients treated with alendronate for 1–8 years. Evidence of impaired fracture healing was observed in six of their patients. The bone biopsies obtained in all patients showed a severe depression of bone formation with an absence of double-tetracycline labelling. Goh et al.[11] were the first to document fractures occurring in the subtrochanteric region of the femur in nine patients who had been taking alendronate. These reports were followed by three additional case series of patients presenting with a low-energy subtrochanteric or proximal femoral shaft fracture from Kwek et al.[12] (16 patients taking alendronate therapy and one taking risedronate after switching from alendronate), Lenart et al.[13] (15 patients taking alendronate) and Neviaser et al.[14] (25 patients taking alendronate). Our patients exhibited fracture patterns (below the lesser trochanter and above the distal one-third of the diaphysis) that were very similar to those reported by Neviaser et al.[14] This fracture pattern is probably a rare complication of bisphosphonates, which could explain why this adverse reaction had not been observed in the long-term studies of Black et al.[22] and Bone et al.[23] Coadministration of estrogens or corticosteroids seems to be a predisposing factor as suggested by Odvina et al.[10] Four of our patients were on corticosteroid therapy and two on postmenopausal hormonal treatment. Further investigation is needed to identify factors that increase the risk of such complications in order to define the proper balance between benefits and potential risks of bisphosphonate treatment.[8]

Bisphosphonates are the most widely used antiresorptive agents for the treatment of diseases involving an increased activity of osteoclasts.[24] Bisphosphonates can be classified into two major groups with different mechanisms of action. The first group comprises the non-nitrogen-containing bisphosphonates (e.g. clodronate, etidronate) which closely resemble pyrophosphate.[4,24] These compounds can be metabolically incorporated into nonhydrolyzable analogues of adenosine triphosphate. The accumulation of these metabolites in the cytosol of osteoclasts results in the inhibition of osteoclast function and may cause cell death. Induction of osteoclast apoptosis seems to be the primary mechanism by which non-nitrogen-containing bisphosphonates inhibit bone resorption. Nitrogen-containing bisphosphonates (e.g. alendronate, pamidronate, ibandronate) act by inhibiting farnesyl diphosphate (FPP) synthase, a key enzyme of the mevalonate pathway.[4,24,25] FPP is required for post-translational modification

(prenylation) of proteins, including small GTPases. GTPases are important signalling proteins that positively regulate several structural properties and cell processes important for osteoclast function. Inhibition of these processes by bisphosphonates leads to an inhibition of bone resorption.

The effect of bisphosphonates on osteoclasts has recently been assessed by Weinstein et al.[26] These authors observed an increase in the number of osteoclasts in bone biopsies from patients receiving alendronate, in contrast to animal studies that showed a decreased number of osteoclasts. Giant osteoclasts with pyknotic nuclei were found in some of the biopsies. Histological data were unfortunately available for only two patients in our study, and no osteoclasts were visible. However, Weinstein et al.[26] reported giant osteoclasts in only 25–56% of the patients treated with alendronate, which could explain our findings. Their observations suggest that the mechanism by which bisphosphonates inhibit bone resorption is still not fully understood and underscore the need for more investigation on the effect of these drugs on human osteoclasts.[27]

Bisphosphonates reduce fracture rates in part by reducing bone turnover.[28] As 'bone hardeners',[29] they produce increases in BMD, sometimes used as a surrogate marker of fracture risk.[30] However, measurements of surrogate markers alone may not be reliable to assess a decrease in fracture incidence.[31] In our study, BMD of the femoral neck could be obtained for four patients, but for two of them the BMD evaluation was performed several years before the first fracture occurred.

By slowing bone turnover, bisphosphonates allow secondary mineralization to progress, thereby increasing the tissue mineral content. The potential harmful effect on bone strength resulting from an inhibition of bone turnover is an important issue.[32] Increased mineralization and accumulation of microdamage are two separate but related consequences of bone turnover inhibition. The higher the tissue mineral content, the stiffer bone becomes, tolerating more peak stress. However, highly mineralized and homogeneous bone can become brittle and less tough, which may lead to the development of microdamages.[28] Microscopic cracks occur in normal bone after stresses encountered in day-to-day life.[32] They are physiologically detected by osteocytes leading to the initiation of a bone-remodelling repair of the damage. If bone resorption is inhibited, this physiological process is impaired and the natural repair of lesions cannot take place, eventually leading to their local extension. Mashiba et al.[33] reported a negative association between turnover and microdamage, although a direct causal relationship of low bone turnover to increased microdamage levels could not be demonstrated by their data. The effects of bisphosphonates on bone mechanical properties were investigated by Yang et al.[34] in a rat osteoporosis model. These authors showed that high concentrations of pamidronate in the bone were associated with a decrease in the mechanical strength of the intact femur.

Most of our patients were receiving alendronate therapy, but three of them developed a fracture after they had been switched to ibandronate therapy. As they previously received long-term alendronate (for 3–10 years), we cannot exclude that the fracture might have been related to a sustained effect of alendronate even after its discontinuation. Kwek et al.[12] also reported the case of a patient who was switched from alendronate to risedronate. From a pharmacokinetic point of view, alendronate has been reported to have a skeletal half-life of 10.9 years.[35] No other bisphosphonate pharmacokinetic studies report such long follow-up.[25] Unlike most other drugs, bisphosphonates remain in the body for decades.[32] They are not metabolized, but either excreted by the kidney or deposited within the bones, and the amount of drug within the bone will accumulate over time. This long-term skeletal half-life has to be considered when assessing the patients' follow-up after discontinuation of bisphosphonate therapy. One of our patients presented with a femur fracture 1 year after discontinuation of pamidronate therapy. A similar observation with alendronate has also been made by Armamento-Villareal et al.[8] Our patient is the only case of low-energy fracture that has been observed with

pamidronate. Although the data are still limited, our cases and those of Kwek et al.[12] suggest that the risk of a low-energy femoral fracture might be a class effect of bisphosphonate. This effect has thus far only been described with alendronate. The fact that this molecule has been available for the longest time and is the most widely prescribed may explain why this complication has to date not been linked to the use of other bisphosphonates.[14]

In the WHO database, 295 cases of fracture have been reported with alendronate thus far (data extracted on 24 July 2008) of 33 244 adverse drug reactions reported with this compound. These cases do not include ours. A majority of these 295 cases were unspecified fractures (n = 243), and there were 23 cases of impaired fracture healing, 27 cases of pathological fractures, one case of spontaneous fracture and one case of osteoporotic fracture. Few cases of such adverse drug reactions have been reported with other bisphosphonates in the WHO database, except for pamidronate, as shown in table III. This database of the WHO Programme for International Drug Monitoring contains spontaneous reports of adverse reactions from member countries, describing suspicions that have arisen from observation of an unexpected or unwanted event. In most instances it cannot be proven that a pharmaceutical product or ingredient is the cause of an event. The volume of reports for a particular product may be influenced by the extent of use of the product, publicity and other factors. No information is provided on the number of patients exposed to the product.[36] The extracted data for a given drug must be taken in the context of a spontaneous reporting system. In any case, these data cannot be used to calculate an incidence rate.

It is interesting to point out that all of our patients except one were receiving long-term treatment with a PPI (mostly esomeprazole). Limited animal and human studies have shown that PPI therapy may decrease insoluble calcium absorption or bone density. A recent case-control study found an association between the long-term use of PPI therapy and an increased risk of hip fracture.[37] The adjusted odds ratio (OR) for hip fracture associated with more than 1 year of PPI treatment was 1.44 (95% CI, 1.30, 1.59). The results of two recently published studies were consistent with these findings.[38,39] The first study[38] analysed data from prospective cohorts of men and women over the age of 65 who were enrolled in the MrOS (Osteoporotic Fractures in Men Study)[40,41] and the SOF (Study of Osteoporotic Fractures)[42] to examine the association between acid-suppressive medication use and bone density, rates of hip bone loss and fracture risk. Analysis of fracture outcomes showed that the use of PPIs conferred a 34% increased risk of nonspine fracture in the cohort of women. Among men who were not taking calcium supplements, there was a 49% greater risk of nonspine fracture compared with those not taking PPIs. The second study,[39] which included more than 15 000 cases of fracture (vertebra, wrist or hip), found a significant association between more than 7 years use of PPI and any assessed fracture (adjusted OR 1.92; 95% CI 1.16, 3.18). The fact that three large, well designed studies consistently report an association between the use of PPI and fractures is a strong basis for encouraging further investigation.[43]

Table III. Bisphosphonate adverse drug reactions (ADRs) reported in the WHO database (until 24 July 2008)

Compound	Total number of ADRs	Total number of fractures	Unspecified fractures	Fracture healing impaired	Fracture pathological	Fracture osteoporotic	Fracture spontaneous
Alendronate (since 1995)	33 244	295	243	23	27	1	1
Risedronate (since 1991)	5 788	31	26	0	4	0	1
Ibandronic acid (since 1996)	633	6	4	0	2	0	0
Ibandronate sodium (since 2006)	5 490	14	14	0	0	0	0
Pamidronate (since 1986)	7 901	50	26	2	22	0	0

A possible mechanism to explain this increase in fracture risk may be via the decreased absorption of calcium among those taking acid suppressive medication. An acidic environment in the gastrointestinal tract facilitates the release of ionized calcium from insoluble calcium salts. Calcium solubility may be important for its absorption. An acid suppressive therapy could therefore hinder calcium absorption. Consequently, potential bone loss may occur if acid production is suppressed. Nevertheless, it is unclear whether calcium malabsorption is sufficiently severe to influence bone modelling, and long-term studies on calcium malabsorption and the negative effects on skeletal metabolism are lacking.[43] All of our patients were receiving calcium supplementation. Calcium values could be obtained for four of our patients. Although we cannot make conclusion from such limited data, it is interesting to note that in two cases, the calcium value within the year of the fracture was below the normal values.

The simultaneous administration of bisphosphonate and PPIs could lead to a potentialization of their respective negative effect on bone strength and thus to an increased risk of fracture. This negative pharmacodynamic interaction is a novel hypothesis that should be investigated by mechanistic and epidemiologic studies.

In addition to bisphosphonates and PPIs, four of our patients were also receiving oral or inhaled corticosteroids. The administration of oral glucocorticoids is associated with a significant increase in fracture risk at the hip and spine.[44] The concomitant use of three drugs having potential negative long-term effects on bone could increase the risk of fracture, which could explain why such a rare adverse effect had not been detected in previous studies.

The retrospective design and the lack of a control group constitute the main limitations of our study. After drug marketing, case reports and case series raise hypotheses about drug effects, but more rigorous study designs are mandatory to test these hypotheses.[45] Although there have been several reports indicating that the long-term use of bisphosphonates may be associated with insufficiency fracture of the femur, we cannot make robust conclusions from case series. Prospective large-scale studies with long-term follow-up are warranted to address the specific question of this rare adverse drug reaction.

Conclusion

We report on a number of patients who developed low-energy or spontaneous fractures of the femur (subtrochanteric or femoral shaft) while on long-term bisphosphonate therapy. This observation might be a class effect of bisphosphonates. Moreover, we are the first to report the concomitant long-term use of PPIs in most of our patients, which could have contributed to the increased risk of fracture. Given the seriousness and the rarity of these cases, we have reported them to the National Health Authorities and consequently to the WHO Collaborating Centre for International Drug Monitoring as well as to the manufacturers of the drugs. Although we cannot draw a conclusion from our case series and other published reports, we believe that prescribers and users of these drugs should be alert to the possibility of such rare adverse reactions. We invite every prescriber of bisphosphonates and PPIs who has patients presenting with similar fracture patterns to report the case to the pharmacovigilance authorities.

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Introduction to the article

“Ing Lorenzini K, Daali Y, Fontana P, Desmeules J, Samer C. Rivaroxaban-Induced Hemorrhage Associated with ABCB1 Genetic Defect. Front Pharmacol. 2016 Dec 19;7:494.”

This article describes a patient presenting with severe anemia related to gastrointestinal bleeding associated with rivaroxaban therapy. He was finally hospitalized for non-ST segment elevation myocardial infarction (NSTEMI). In his case, several causes were identified as risk factors for rivaroxaban overdose, pharmacogenetics polymorphisms, drug-drug interaction, and acute renal failure. Genotyping showed that the patient was a homozygous carrier of two *ABCB1* variant alleles, c.2677G>T TT and c.3435C>T TT. These alleles have been associated with a lower functionality of the encoded transporter, P-glycoprotein (P-gp), which is involved in the pharmacokinetics of rivaroxaban, as well as of other direct oral anticoagulants (i.e. dabigatran, edoxaban, apixaban). We hypothesized that reduced P-gp functionality led to an accumulation of rivaroxaban, thereby increasing the risk of ADR such as bleeding. Moreover, at the time of gastrointestinal hemorrhage, the patient was taking simvastatin, a known inhibitor of cytochrome P450 3A4/5 (CYP), an enzyme involved in rivaroxaban metabolism. Our article underscores the importance of individual case safety reports in generating hypotheses not always thoroughly studied during clinical drug development such as the impact of pharmacogenetics.

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CASE REPORT
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Rivaroxaban-Induced Hemorrhage Associated with *ABCB1* Genetic Defect

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We report a patient who presented a non-ST segment elevation myocardial infarction in the context of severe normocytic hypochromic anemia related to gastrointestinal bleeding, 3 months after switching anticoagulant from the vitamin K antagonist acenocoumarol to the direct oral anticoagulant rivaroxaban. High levels of both anti-Xa activity and rivaroxaban plasma concentrations were measured despite rivaroxaban withdrawal, suggesting reduced elimination/drug clearance. Estimated half-life was 2–3 times longer than usually reported. The patient is a homozygous carrier of *ABCB1* variant alleles, which could have participated to reduced elimination of rivaroxaban. Furthermore, CYP3A4/5 phenotyping showed moderately reduced enzyme activity. Drug-drug interaction with simvastatin may have contributed to decreased rivaroxaban elimination. Although in the present case moderate acute renal failure probably played a role, more clinical data are required to elucidate the impact of *ABCB1* polymorphism on rivaroxaban pharmacokinetics and bleeding complications.

Keywords: direct oral anticoagulants, adverse drug reaction, genetic polymorphism, *ABCB1*, CYP3A4/5, drug-drug interaction

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INTRODUCTION

Rivaroxaban is a direct Factor Xa inhibitor which has been approved for specific thromboembolic disorders such as the prevention of stroke and systemic embolism in adults with non-valvular atrial fibrillation (Mueck et al., 2014). Rivaroxaban is both excreted as unchanged drug in urine and through metabolic transformation. P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), as well as cytochrome P450 (CYP) 3A4/5 have been shown to play major roles in rivaroxaban transport and metabolism, respectively (Mueck et al., 2014). The administration of strong inhibitors of CYP3A4/5 and P-gp/BCRP such as ritonavir and ketoconazole has been shown to increase rivaroxaban exposure and cases of severe bleeding have been reported (Mueck et al., 2013). However, to the best of our knowledge, no human data has described the impact of genetic polymorphism of *ABCB1* and/or *ABCG2* on the pharmacokinetics and safety of rivaroxaban. *ABCB1* and *ABCG2* genes encode for P-gp and BCRP efflux transporter, respectively, (Hodges et al., 2011; Giacomini et al., 2013). We report here a rivaroxaban-treated patient who presented with severe anemia related to gastrointestinal bleeding and in whom *ABCB1* genetic polymorphism and drug-drug interaction (DDI) may have been contributing factors. The patient gave his written informed consent for publication of this report.

CASE PRESENTATION

Our patient is a 79-year-old male suffering from systolic cardiac failure (ischemic, rhythmic, and valvular) and type 2 diabetes mellitus. The patient had received rivaroxaban 20 mg q.d. since September 2015 for cardioembolic strokes and atrial fibrillation. Before the introduction of rivaroxaban, he had been treated with acenocoumarol for years. The patient was hospitalized on December 15th 2015 for non-ST segment elevation myocardial infarction (NSTEMI). At hospital admission, laboratory testing showed severe normocytic hypochromic anemia with a hemoglobin level at 70 g/l (normal range: 140–180 g/l), without hemodynamic instability. The patient received erythrocyte transfusions, which raised the hemoglobin to 105–110 g/l. Acute renal failure was also diagnosed with a CL_{CR} value at 39 ml/min using the Cockcroft–Gault equation at admission. Renal function improved at 57 ml/min 4 days later. Due to the presence of fecal occult blood on two occasions, iron loss from gastrointestinal bleeding was suspected. The colonoscopy did not show any evidence of colon injury; however, inadequate bowel preparation was highlighted by the examinator. Gastroscopy could not be performed because the patient's comorbidities exposed him to high risks in case of general anesthesia. Rivaroxaban was stopped at admission; enoxaparin was introduced 4 days later and then switched to acenocoumarol.

The other patient medications before hospitalization were: insulin, simvastatin 40 mg q.d., levothyroxine 75 μg q.d., extended-release metoprolol 25 mg q.d., and enalapril 10 mg q.d.

INVESTIGATIONS

Clinical investigations were performed to assess for causes of potential increased rivaroxaban effects at therapeutic doses. They included anti-Xa activity measurement, rivaroxaban plasma concentrations measurement, as well as *ABCB1* genotyping, and CYP3A4/5 phenotyping.

Anti-Xa Activity

Anti-Xa activity was measured with a chromogenic assay using the DiXal® kit (Hyphen Biomed, Neuville-Sur-Oise, France) and a BCS XP instrument (Siemens, Marburg, Germany). This method has a limit of detection of 10 ng/ml. No information is given by the manufacturer regarding the limit of quantification (LOQ). However, previous studies have shown a LOQ of 20–30 ng/ml (Douxfils et al., 2013). The accuracy and precision calculated from the quality controls (QCs) were 107.0 and 8.8%, respectively, (Asmis et al., 2012). An excellent correlation between this method and liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been shown (Spearman correlation coefficient of 0.96) (Douxfils et al., 2013).

Rivaroxaban Plasma Concentrations

Rivaroxaban determination was performed using a fully validated LC-MS/MS method according to guidelines of the US Food and Drug Administration and the International Conference on Harmonization. The method was accurate and precise across the dynamic range of 0.5–1000 ng/ml. The LOQ was 0.5 ng/ml. The mean precision and accuracy, calculated from the QCs, were 10.2 and 112%, respectively.

A plasma sample of 40 μl was processed by protein precipitation extraction using acetonitrile (200 μL). Separation was performed on a C18 column (50 mm × 2.1 mm ID; 2.6 μm particle size) and under gradient conditions using formic acid 10 mM in water and formic acid 10 mM in acetonitrile. Detection was by tandem-MS in positive mode using a Qtrap API 6500 from AB sciex (Ontario, Canada) using rivaroxaban-d4 as internal standard (20 ng/ml).

ABCB1 Genotyping

Genomic DNA was extracted from whole blood (200 μl) using the QIAamp DNA blood mini kit (QIAGEN, Hombrechtikon, Switzerland). *ABCB1* c.3435C>T and c.2677G>T polymorphisms were determined in a single multiplex PCR, with fluorescent probe melting temperature analysis on a LightCycler (Roche, Rotkreuz, Switzerland) as previously described (Ansermot et al., 2008).

CYP3A4/5 Phenotyping

Midazolam was used as a probe to measure the joint activity of CYP3A4/5 as previously described (Bosilkovska et al., 2014). Phenotyping was performed 8 days after hospital admission with concomitant treatment of insulin, enoxaparin 60 mg b.i.d., atorvastatin 40 mg q.d. (replacing simvastatin from the day of hospital admission), esomeprazole 40 mg q.d., levothyroxine 75 μg q.d., lisinopril 10 mg q.d., extended-release metoprolol 50 mg q.d., picosulfate 5 mg q.d., and spironolactone 25 mg q.d.

RESULTS

Results from anti-Xa activity and rivaroxaban plasma concentrations are presented in **Table 1**. The patient was a homozygous carrier of both tested *ABCB1* variant alleles. His genotype was TT for the c.2677G>T single nucleotide polymorphism (SNP) and TT for the c.3435C>T SNP. CYP3A4/5 phenotyping showed moderately decreased enzymatic activity, with OH-midazolam/midazolam metabolic ratio of 0.31.

TABLE 1 | Anti-Xa activity and rivaroxaban plasma concentrations.

Time after the last rivaroxaban dose	Anti-Xa activity (ng/ml)	Rivaroxaban plasma concentrations (ng/ml)
24 h	231	not performed
48 h	110	88.3
60 h	81	not performed
72 h	66	70.5
84 h	66	not performed
96 h	34	35.6

DISCUSSION

We described the case of a rivaroxaban-treated patient who presented a non-ST segment elevation myocardial infarction in the context of a severe anemia probably due to a gastrointestinal bleeding, 3 months after switching anticoagulant treatment from acenocoumarol to rivaroxaban. Laboratory investigations showed high levels of anti-Xa activity and rivaroxaban plasma concentrations at trough level (24 h after the last dosing) and an unexpected delay for rivaroxaban clearance, suggesting impaired rivaroxaban elimination. Our hypothesis is that both genetic and environmental factors might have contributed to an increased susceptibility to rivaroxaban in this patient, e.g., the homozygous presence of *ABCB1* variant alleles and reduced CYP3A4/5 activity due to DDI with simvastatin. Given that more than one third of the dose is eliminated as unchanged active drug in the urine (Mueck et al., 2014), acute renal failure was probably also a contributing factor. However, renal impairment at admission was moderate (39 ml/min), not requiring dose adjustment according to the summary of product characteristics. In a physiologically based pharmacokinetic (PBPK) model simulating the combined effect of renal impairment and concomitant erythromycin (combined P-gp and moderate CYP3A4/5 inhibitor) administration on rivaroxaban pharmacokinetics, concurrent renal impairment plus erythromycin resulted in a 2.5–3 fold increase in rivaroxaban exposure in elderly (Grillo et al., 2012). However, *in vivo* data showed that the impact of concomitant renal impairment and erythromycin administration was less than expected by the PBPK model. Indeed, a clinical study showed that in subjects with moderate renal impairment receiving concomitant erythromycin, rivaroxaban AUC, and Cmax values increased by approximately 99 and 64%, as compared with subjects with normal renal function receiving rivaroxaban 10 mg alone (Moore et al., 2014).

Rivaroxaban belongs to the recently developed class of direct oral anticoagulants (DOACs). Older OACs such as vitamin K antagonists (VKAs) are characterized by extensive hepatic metabolism by CYP450, narrow therapeutic window and the need for routine coagulation monitoring for dose adjustment. Their extensive hepatic metabolism implies multiple potential DDI and an impact of CYP2C9 genetic polymorphism on their pharmacokinetics (Scaglione, 2013). DOACs are given at fixed doses without the need for routine monitoring (Mueck et al., 2014). However, these compounds are also subject to DDI due to CYP450-mediated metabolism and/or P-gp and/or BCRP-mediated transport (Scaglione, 2013). About one third of a rivaroxaban dose is renally excreted as an unchanged form (Mueck et al., 2014) via glomerular filtration and tubular secretion (Scaglione, 2013). P-gp and BCRP have been shown to be the major transporters involved in the active renal secretion of rivaroxaban (Mueck et al., 2014). The remaining part of the dose is eliminated after hepatic metabolism. CYP3A4/5 and CYP2J2 are the isoenzymes involved in rivaroxaban metabolism, while CYP-independent mechanisms are also contributing (Mueck et al., 2014).

To the best of our knowledge, no human data have yet described the impact of *CYP3A4/5* and/or *ABCB1 (MDR1)* and *ABCG2* gene polymorphism on the pharmacokinetics and safety of rivaroxaban. More than 100 single nucleotide polymorphisms (SNPs) of the *ABCB1* gene region occurring at a >5% frequency have been described. For the most common coding SNPs (c.2677G>T, c.3435C>T), allele frequencies exhibits large interethnic differences, ranging between 2 and 90% across populations and depending on the SNP (Hodges et al., 2011). These SNPs are in high linkage disequilibrium and are therefore observed most frequently as haplotypes. In the Caucasian population, the TT TT haplotype frequency ranges from 15 to 25% (Marzolini et al., 2004).

An animal study showed that Mdr1a/Mdr1b/Bcrp triple knockout mice exhibited higher rivaroxaban plasma levels than wild type mice, with a 1.7 fold increase in plasma concentration 4 h after administration (Gong et al., 2013). The observed effect was explained by decreased excretion of rivaroxaban via P-gp and/or BCRP rather than increased absorption as rivaroxaban oral bioavailability is high (80–100%; Mueck et al., 2014). *ABCB1* genotype of our patient was a homozygous carrier for both tested SNP (c.2677G>T: TT; and c.3435C>T: TT). This genotype might have contributed to the high levels of anti-Xa activity and rivaroxaban plasma concentrations measured. Indeed, levels of anti-Xa activity as determined by a chromogenic assay were higher than expected. The trough level of anti-Xa activity measured 24 h after last drug administration (231 ng/ml) was in the range of expected Cmax levels (Groupe de travail RivaMoS Suisse, 2013). The level measured 4 days after last drug administration (34 ng/ml) was in the range of expected trough levels (Groupe de travail RivaMoS Suisse, 2013). Rivaroxaban plasma concentration measured by LC/MS-MS 48 h after administration (88 ng/ml) was comparable to approximately 8–16 h post-dose predicted levels based on a population pharmacokinetic model (20 mg q.d. administration) (Mueck et al., 2011). The plasma concentration measured 4 days after last drug administration (36 ng/ml) was comparable to expected trough concentrations. The estimated half-life was increased by threefold in our patient, approximately 24–30 h as compared to 11–13 h in the literature for old patients (Mueck et al., 2014).

Drug-Drug Interaction probably also played a role in rivaroxaban high levels. Crossover studies in healthy volunteers have shown significant increases in rivaroxaban systemic exposure during concomitant administration with strong inhibitors of CYP3A4/5 (and possibly CYP2J2), P-gp and BCRP. Steady state administration of ketoconazole led to a dose-dependent increase in rivaroxaban AUC and Cmax. AUC and Cmax increased by, respectively, 82% (90% CI 59%, 108%) and 53% (90% CI 27%, 85%) with ketoconazole 200 mg q.d. compared with rivaroxaban alone. With a higher dose of 400 mg q.d., the measured increases in AUC and Cmax were 158% (90% CI 136%, 182%) and 72% (90% CI 61%, 83%) (Mueck et al., 2013). As a consequence, the concomitant use of rivaroxaban with strong CYP3A4/5 and P-gp/BCRP inhibitors should be avoided due to a potential increase in the risk of bleeding (Mueck et al., 2013). According to the data from the ROCKET AF study

(Prevention of Stroke and Embolism Trial in Atrial Fibrillation), the presence of combined CYP3A4/5 and P-gp inhibitors did not have any impact on safety outcomes such as bleeding events when comparing the rivaroxabanand warfarin groups (Piccini et al., 2016). However, key exclusion criteria included use of a strong CYP3A4/5 inhibitors or inducers. As recently underscored by Bouatou et al. (2016) this represents a major bias when analyzing the impact of polypharmacy pharmacokinetic interactions on the efficacy and safety of rivaroxaban. Moreover, the prescription of P-gp affecting drugs in heart failure patients is as frequent as 40–50% as shown by Jungbauer et al. (2010).

In the present case, our patient was receiving long-term treatment with simvastatin. Recently, an *in vitro* study has shown that simvastatin inhibited CYP3A4/5 and P-gp activity (Lee et al., 2015). An *in vivo* animal study performed by the same authors showed that concomitant administration of simvastatin with nifedipine, a CYP3A/5 and P-gp substrate, significantly increased the absolute bioavailability of nifedipine by 150% (Lee et al., 2015). On the other hand, atorvastatin inhibited CYP3A/5 but not P-gp activity *in vitro*, and had no impact on nifedipine pharmacokinetics *in vivo* (Lee et al., 2015). According to the FDA classification[1], simvastatin would therefore be considered as a weak CYP3A/5 inhibitor ($\geq$1.25 but <2-fold increase in AUC). CYP3A4/5 phenotyping, which was performed 8 days after the patient had been switched from simvastatin to atorvastatin, showed moderately decreased enzymatic activity as compared to our study population of healthy volunteers (Bosilkovska et al., 2014), which could be attributed at least in part to inhibition by atorvastatin. Although the enzymatic activity was only modestly decreased with a OH-midazolam/midazolam metabolic ratio of 0.31, compared to 0.50 in healthy volunteers without enzyme inhibitor/inducer, and 0.22 in the presence of a CYP inhibitor (voriconazole) (Bosilkovska et al., 2014), the measured phenotype allowed to rule out an ultrarapid metabolism.

The present case has some limitations. We did not investigate *ABCG2* and *CYP2J2* gene polymorphism. Indeed, CYP2J2 is also subject to genetic variations and the promoter region SNP (CYP2J2-76G>T; *7 allele) reportedly decreases epoxygenase activity *in vivo* (Murray, 2016). Moreover, we did not evaluate P-gp activity *in vivo* through phenotyping and the DDI with simvastatin has not been thoroughly investigated. Finally, the moderate acute renal failure at admission was probably a contributing factor to rivaroxaban high levels.

[1] http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm

CONCLUDING REMARKS

Our patient presented severe normocytic hypochromic anemia probably due to gastrointestinal bleeding, 3 months after switching his anticoagulant treatment from acenocoumarol to rivaroxaban.

Laboratory investigations showed high levels of anti-Xa activity and rivaroxaban plasma concentrations after rivaroxaban withdrawal, suggesting reduced rivaroxaban elimination (estimated half-life: 24–30 h). We suggest that the homozygous presence of *ABCB1* variant alleles and possibly altered CYP3A4/5 activity due to DDI with simvastatin were possible contributing factors, in addition to the moderate decreased renal function.

More clinical data are required to elucidate the impact of genetic polymorphism on rivaroxaban pharmacokinetics and bleeding complications. The impact of *ABCB1*, *ABCG2,* and *CYP2J2* gene polymorphism on rivaroxaban pharmacokinetics should be investigated in a phase 1 clinical study in healthy volunteers. The impact of genetic polymorphism on the susceptibility to DDI should also be further investigated. Indeed, the presence of a variant allele could predispose to DDI, as suggested between warfarin and simvastatin (Andersson et al., 2012).

AUTHOR CONTRIBUTIONS

KIL was in charge of the pharmacological investigations done in the patient, interpreted the results, and wrote the manuscript. YD measured the rivaroxaban plasma concentrations and performed the phenotyping test. PF was involved in the care of the patient and was in charge of the anti-Xa measurements and of interpretation of the results. JD supervised the investigations and the redaction of the manuscript. CS interpreted the results and wrote the manuscript. All authors read and approved the manuscript.

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Conflict of Interest Statement: 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.

Part 3

Postmarketing studies for drug safety surveillance

Introduction to the article

“Ing Lorenzini K, L'Huillier AG, Crisinel PA, Rebsamen MC, Fluss J, Korff CM, Barbe RP, Siegrist CA, Dayer P, Posfay-Barbe KM, Desmeules JA; H1N1 Pediatric Epidemiology Study Group of Geneva. ABCB1 polymorphisms and neuropsychiatric adverse events in oseltamivir-treated children during influenza H1N1/09 pandemia. Pharmacogenomics. 2011 Oct;12(10):1493-501.”

This article describes a prospective cohort study conducted during the H1N1/09 pandemia which primarily aimed at assessing the safety profile of oseltamivir in children. Our secondary objective was to evaluate the association between oseltamivir-induced neuropsychiatric adverse events (NPAEs) and *ABCB1* genetic polymorphism. NPAEs have indeed been reported in children taking this antiviral drug. Oseltamivir is a P-gp substrate, which limits its penetration through the blood-brain barrier. We hypothesized that children carrying *ABCB1* variant alleles may be more prone to NPAEs through better central nervous system penetration. Among 109 children enrolled in the study, 54 H1N1 positive children received oseltamivir treatment and 42 had pharmacogenetic analyses. Adverse events were systematically recorded, regardless of severity, either by pediatricians during hospitalization, and/or by parents at home using a diary card. More than half of our patients (60%, 25/42) reported at least one adverse event associated with oseltamivir treatment, and 36% (15/42) displayed NPAEs, mainly nightmares, agitation, and irritability. Although the association between genetic polymorphism and the occurrence of NPAE was not significant, we observed that the frequency of NPAE displayed a ‘genotype-trend effect’ with the variant and the wild-type subgroups at the two far ends. Wild-type homozygous presented much less NPAE compared with variant homozygous (11 vs 67%), while heterozygous individuals for at least one variant were in between (39%). Our article underscores the importance of postmarketing studies to study drug safety in orphan populations such as children and to evaluate the impact of pharmacogenetics.

ABCB1 polymorphisms and neuropsychiatric adverse events in oseltamivir-treated children during influenza H1N1/09 pandemia

Aims: To examine the safety profile of oseltamivir in children and evaluate the impact of P-glycoprotein polymorphisms on the incidence of neuropsychiatric adverse events (NPAE) in oseltamivir-treated children. **Subjects & methods:** This prospective cohort study was conducted in our tertiary care pediatric hospital (University Hospitals of Geneva, Switzerland) during the H1N1 pandemia, between 1 October 2009 and 31 January 2010. All newborn to 18 year-old patients presenting at the emergency department with a flu-like illness were eligible for inclusion. Adverse events were systematically recorded by pediatricians and/or by parents at home using a diary card, with a 30-day follow-up period. The causality assessment of oseltamivir in NPAE was performed by two clinical pharmacologists. After informed consent, enrolled patients were also genotyped for *ABCB1* 3435C>T (rs1045642) and 2677G>T/A (rs2032582) polymorphisms. **Results:** Among the 42 H1N1-infected, oseltamivir-treated children who were genotyped for *ABCB1* 3435C>T and 2677G>T/A variants, 36% presented NPAE. When examining the association between the diplotype and the development of NPAE, we observed that the frequency of NPAE displayed a 'genotype-trend effect' with the variant and the wild-type subgroups at the two far ends. A total of 11% of the 2677GG–3435CC individuals (wild-type homozygous) presented NPAE, compared with 39% of the individuals being heterozygous for at least one variant allele and 67% of the 2677TT–3435TT individuals (homozygous variants) (p = 0.149, nonsignificant). **Conclusion:** These observations suggest a potential influence of *ABCB1* polymorphisms in oseltamivir-related NPAE, maybe as a result of an enhanced permeability of the blood–brain barrier to oseltamivir.

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KEYWORDS: adverse events ▪ anti-infective ▪ pediatrics ▪ pharmacogenetics ▪ psychiatric

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A new human H1N1 influenza virus first appeared in Mexico in the spring of 2009 [1] and a Phase 6 pandemia was declared by the WHO on 11 June of the same year. In Switzerland the epidemic threshold was reached during week 43, peaked at week 49, and lasted until the end of March 2010.

According to national recommendations published in August 2009 [101], oseltamivir, an inhibitor of neuraminidase, was used to treat potentially infected patients at risk of complications (including infants under 12 months), those with severe symptoms or those in close contact with at-risk patients [102]. Oseltamivir is a prodrug that is readily absorbed from the GI tract and extensively converted to the active metabolite oseltamivir carboxylate (OC) by hepatic carboxylesterases [2]. Two recent reviews summarize the current knowledge of the pharmacokinetic characteristics of oseltamivir [3,4], one of them focusing on pediatric data [4]. The pharmacokinetics of oseltamivir carboxylate after oral administration of oseltamivir is characterized by mean bioavailability of 79% [3]. The extent of exposure has been shown to be reduced when oseltamivir was dissolved in milk. It is therefore plausible that the relative peroral bioavailability of oseltamivir is reduced in neonates and young infants receiving regular feeds with human milk and/or milk-based infant formulas compared with adults [4]. OC is largely eliminated through the kidneys. Approximately 60–70% of an oral dose appears in the urine as the active metabolite, and less than 5% as oseltamivir. Values reported for the apparent oral clearance of the renally eliminated OC are in excess of glomerular filtration, suggesting that elimination occurs via filtration and active tubular secretion. The average elimination half-life of OC varied around 7.4 h [3]. Published pharmacokinetic data in children show a reduced bioavailability and an expanded distribution volume in neonates and young infants coupled with enhanced renal clearance in infants and young children. The half-life of OC appears to demonstrate a degree of age-dependence (<2 years: 14.9 h; 3–5 years: 11.3 h 13–18 years: 8.1 h) [4].

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Oseltamivir was generally well-tolerated during clinical studies involving children, the most frequently reported adverse event being vomiting (approximately 15% of children) [5]. The majority of reports of neuropsychiatric adverse events (NPAE) (including delirium, convulsions and encephalitis) in children taking oseltamivir have mainly originated from Japan [6]. The precise mechanism is not known; the events could be caused by oseltamivir, OC, or by the disease itself [7].

Animal studies have demonstrated that oseltamivir penetrates through the blood–brain barrier, but that P-glycoprotein (P-gp) limits its uptake in the brain [8,9]. Indeed, a pharmacokinetic study in healthy human volunteers showed a low penetration of oseltamivir into the cerebrospinal fluid, as well as of OC [10]. P-gp, the encoded product of the human *ABCB1* (*MDR1*) gene, is a membranous transporter, which is expressed at the luminal membrane in brain capillaries [11] and which presents several genetic SNPs. Variants in exons 26 (3435C>T) and 21 (2677G>T/A) are the most extensively studied and are associated with differences in expression and/or function [12,13]. *In vitro* and *in vivo* studies on P-gp over-expressing cells and on MDR1a/1b knock-out mice showed that oseltamivir, but not OC, is a substrate of P-gp [8,9]. The carboxylate metabolite has been shown to be a substrate MRP4/*ABCC4* and of the OAT3/*SLC22A8*. The MRP4 is an ATP-binding cassette transporter localized in brain capillary endothelial cells that accepts anionic drugs as substrates. The OAT3 is a transporter which is expressed on the abluminal membrane of the brain capillary endothelial cells in rodents, but its functional importance at the human blood-brain barrier has not yet been established. The OAT3 may act in the efflux of OC from inside the endothelial cells into the brain, as well as in its uptake from the brain to the endothelial cells (bidirectional transport) [14]. Moreover, the transporters MRP4 and OAT3, as well as OAT1, play a role in the renal tubular secretion of OC [3]. The interaction with MRP4, in conjunction with the hydrophilic property of OC results in a low penetration of OC into the CNS. However, given this low permeability, OC formed from oseltamivir by cerebral carboxylesterase could accumulate in the brain. Nonetheless, this conversion of oseltamivir to OC in the brain seems to be limited compared with the liver [15].

During the H1N1 pandemia, we conducted a prospective cohort study in children. Our study was part of a larger study, which concerned 109 patients during the H1N1 pandemia and aimed to compare the clinical presentation of febrile respiratory tract infections in H1N1/09 positive and negative patients [16]. The primary objective of our study was to examine the safety profile of oseltamivir in the pediatric population, with a focus on NPAE. Our secondary objective was to assess if the presence of *ABCB1* 3435C>T and 2677G>T/A variants could increase the risk of NPAE in oseltamivir-treated children.

Subjects & methods

The protocol of this cohort study was approved by the ethics committee of our institution (protocol number: 09-192). The trial was registered prior to patient enrolment at ClinicalTrials.gov (clinicaltrials.gov identifier: NCT01022931) and conducted in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice and Swiss regulatory requirements. Written informed consent was obtained from a parent, and the child whenever possible, prior to participation. From 1 October 2009 to 31 January 2010, newborn to 18 year-old patients presenting at the emergency department of our tertiary care pediatric hospital (University Hospitals of Geneva, Switzerland) with a flu-like illness were eligible for inclusion in the study, according to national recommendations for usage of oseltamivir. Demographical and clinical data were recorded in a standardized questionnaire. Oseltamivir (Tamiflu®, Roche, Reinach, Switzerland) was prescribed at an oral dose of 2–3 mg/kg for infants younger than 12 months, and 30–75 mg (depending on the weight) for older children, twice a day for 5 days, if H1N1 infection was confirmed by PCR. Oseltamivir was stopped in the case of a negative PCR result. Adverse events were systematically recorded, regardless of severity, either by pediatricians during hospitalization, and/or by parents at home using a diary card, with a 30-day follow-up period. To collect the data, parents were systematically contacted by telephone at days 2 and 7, and seen again at day 30 after oseltamivir treatment onset. NPAE (seizures, agitation, irritability, behavior modifications, nightmares, hallucinations, perception disorders and sleeping disorders) were actively elicited using a standardized screening questionnaire, the Safety Monitoring Uniform Report Form developed by the National Institute of Mental Health-sponsored network of the Research Units on Pediatric Psychopharmacology [17], adapted by child psychiatrists and neurologists from our institution. Other recorded adverse events were systematically searched for: gastrointestinal

(nausea, vomiting and diarrhea) and cutaneous (rash and toxidermia). For each adverse event, including NPAE, the following information was recorded: onset, duration, outcome (resolved, persistent, sequelae and death), severity (mild, moderate or severe). The causality assessment of oseltamivir in NPAE was performed by two clinical pharmacologists, using the WHO-Uppsala Monitoring Centre (UMC) system [103]. The clinical pharmacologists were not blinded to the genetic data (*ABCB1* genotype). However, the genetic data were provided by the laboratory once the causality assessment of oseltamivir in adverse events had already been performed.

ABCB1 genotyping was performed in our center's toxicogenetic and molecular clinical chemistry laboratory. Genomic DNA was extracted from whole blood (200 µl) using the QIAamp DNA blood mini kit (Qiagen, Hombrechtikon, Switzerland). *ABCB1* 3435C>T (rs1045642) and 2677G>T/A (rs2032582) polymorphisms were determined in a single multiplex PCR, with fluorescent probe melting temperature analysis on a LightCycler (Roche, Rotkreuz, Switzerland) as previously described [18]. Pairwise linkage disequilibrium analysis was calculated from estimated haplotype frequencies using the SHEsis software [19]. No other genetic polymorphisms were investigated in the study participants.

The number of patients presenting to our hospital during the study period determined the sample size. Given the setting of our study, an *a priori* calculation of sample size was not performed, as the number of children presenting with a flu-like illness could not be planned.

The primary outcome was the systematic assessment of adverse effects, with a focus on NPAE. The secondary outcome was the association between the development of NPAE defined as possibly related to oseltamivir by the clinical pharmacologists and the presence of *ABCB1* 3435C>T and 2677G>T/A variants. We also assessed for a correlation between the development of NPAE, age and gender.

Standard descriptive statistics were used. For group comparison, χ^2 or Fisher's test was used in the case of binary outcomes. p-values < 0.05 were considered statistically significant. Statistical analyses were performed with SPSS statistical software (version 15.0; SPSS Inc., Chicago, IL, USA).

Results

A total of 109 patients were enrolled in the cohort study [16], from which our study was a part of (median age of 7.02 years old, interquartile range [IQR] of 6.99). A total of 69% (75/109) had H1N1 disease confirmed by PCR, of which 72% (54/75) were treated with oseltamivir. Thus, 54 patients were in our study. Among oseltamivir-treated patients, 96% (52/54) responded to our 30-day follow-up questionnaire and 78% (42/54) had a genetic analysis. Figure 1 illustrates the study flowchart. The two patients who did not respond to the questionnaire did not have a genetic analysis. Twelve patients had no genetic analysis owing to parental refusal. Demographics are described in Table 1. Oseltamivir-treated patients were older (p = 0.022), had more risk factors (p = 0.001) and were more frequently receiving antibiotics (p = 0.018) than untreated patients. Risk factors among the oseltamivir-treated patients were asthma or wheezing history in seven, immunosuppression in ten, sickle cell disease in three, cerebral palsy in three and prematurity in one patient. One patient stopped oseltamivir treatment after four days because of adverse gastrointestinal discomfort.

The distribution of *ABCB1* 3435C>T and 2677G>T/A genotypes is described in Table 2. In two patients, the 3435C>T genotype could

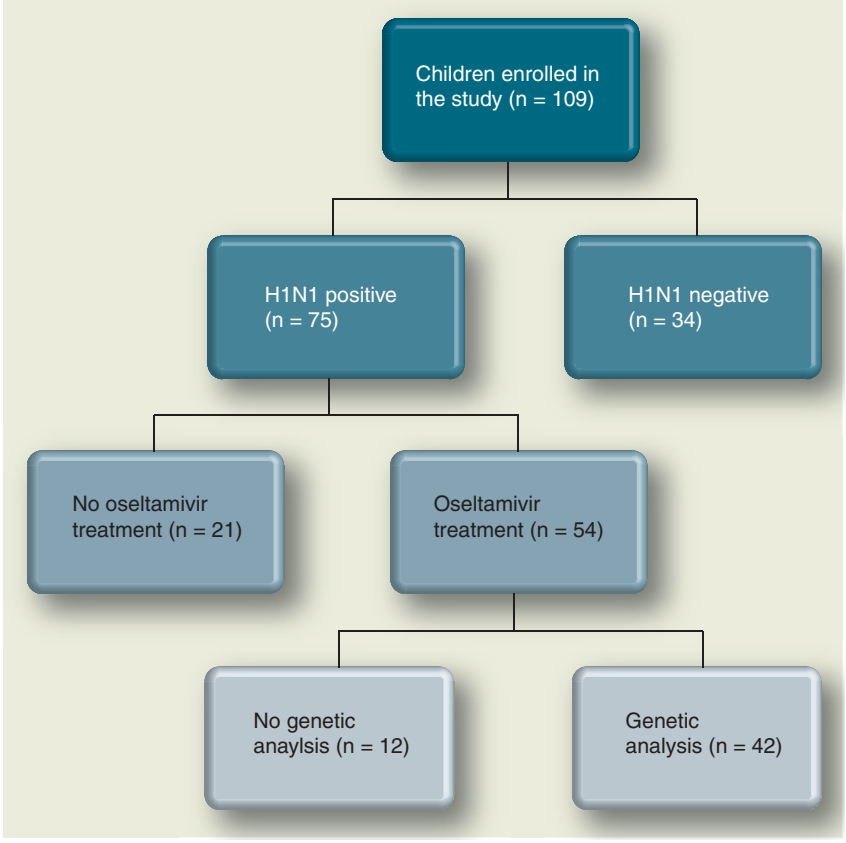

Figure 1. Enrollment of study subjects. Children presenting with a flu-like illness were included in the cohort study. H1N1 positive children who were treated with oseltamivir according to the Swiss recommendations were proposed to participate in the genetic study.

Table 1. Demographics of H1N1-positive patients, according to oseltamivir treatment.

Characteristic	Total (n = 75)	Oseltamivir treatment (n = 54)	No oseltamivir treatment (n = 21)	p-value
Median age, years (IQR)	8.2 (5.9)	9 (7.1)	7.2 (6.1)	0.022
Female gender, % (n)	47 (35)	46 (25)	48 (10)	NS
Caucasian ethnicity, % (n)	73 (55)	72 (39)	76 (16)	NS
Risk factor, % (n)	33 (25)	44 (24)	5 (1)	0.001
Median delay of consultation, days (IQR)	1 (1)	1 (1)	2 (4.5)	NS
Comedication with paracetamol, % (n)	60 (45)	61 (33)	57 (12)	NS
Comedication with NSAIDs, % (n)	37 (28)	41 (22)	29 (6)	NS
Comedication with antibiotics, % (n)	16 (12)	22 (12)	0 (0)	0.018

IQR: Interquartile range; NS: Nonsignificant.

not be determined with the PCR method used in our laboratory. A strong linkage disequilibrium was observed between 3435C>T and 2677G>T/A (D′ = 0.916, coefficient of linkage disequilibrium).

The distribution of adverse events, including NPAE, was similar whether the ten patients without genetic analysis were included or not. Thus, only NPAE in patients with genetic analyses are described (Table 3). A total of 25 out of the 42 patients (60%) reported adverse events with oseltamivir treatment, and 36% (15/42) of the patients presented NPAE, possibly related to oseltamivir. A patient could present several NPAE; indeed three patients presented more than one NPAE. Nightmares were described in 6/15 patients (40%), agitation in six patients (40%), irritability in five patients (33%), sleeping disorders in one patient (7%), and seizures in one patient (7%). After exclusion of sleeping disorders (including patients presenting only nightmares), 21% (9/42) of the treated patients presented NPAE. The majority of NPAE started 12–36 h after the beginning of oseltamivir treatment, and resolved before day 7. No correlation was observed between the development of NPAE, age and gender (p = 0.756 and p = 0.621, respectively). The other adverse events were gastrointestinal symptoms (nausea, vomiting, or diarrhea, n = 9) and rash (n = 1).

Table 4 summarizes the frequency of NPAE according to the presence of variant alleles. For 3435C>T polymorphism, 40% (12/30) of the patients carrying at least one 3435C>T variant allele (CT or TT genotype) presented a NPAE, compared with 20% (2/10) of wild-type patients (wild-type for the 3435C>T polymorphism, CC carriers, p = nonsignificant [NS]). When detailing the results by the number of variant alleles, we found that 22% (2/9) of carriers of two variant alleles (TT genotype) presented a NPAE, compared with 48% (10/21) of carriers of one variant allele (CT genotype, p = NS) and 20% (2/10) of wild-type patients (wild-type for the 3435C>T polymorphism, CC genotype, p = NS). In one of the patient presenting NPAE, the 3435C>T could not be determined.

For 2677G>T/A polymorphism, 38% (10/26) of the patients carrying at least one 2677G>T/A variant allele (GT, GA, TT or TA genotype) presented a NPAE, compared with 31% (5/16) of wild-type patients (wild-type for the 2677G>T/A polymorphism, GG genotype, p = NS). When detailing the results by the number of variant alleles, we found that 50% (2/4) of carriers of two variant alleles (TT or TA genotype) presented a NPAE, compared with 36% (8/22) of carriers of one variant allele (GT or GA genotype, p = NS) and 31% (5/16) of wild-type patients (wild-type for the 2677G>T/A polymorphism, GG genotype, p = NS).

We also examined the association between the development of NPAE and diplotype (Figure 2). Given the small size of our population and the number of possible diplotypes (seven diplotypes), we defined only three groups, 2677GG–3435CC (wild-type homozygous), 2677TT–3435TT (homozygous variants) and a third group consisting of individuals being heterozygous for either 2677G>T/A or 3435C>T or both. A total of 11% (1/9) of the wild-type homozygous presented a NPAE, compared with 39% (11/28) of the heterozygous and 67% (2/3) of the variant homozygous (p = 0.149, NS). This analysis did not include the two patients in which the 3435C>T genotype could not be determined.

Discussion

The distribution of *ABCB1* 3435C>T and 2677G>T/A polymorphisms observed in this study is similar to other reports [12,20]. Conversely, we observed one or more NPAE in

Table 2. Distribution of *ABCB1* 3435C>T and 2677G>T/A genotypes.

Alleles (n)	2677G>T/A			3435C>T			2677–3435 diplotype			
	Genotype	*%*	*n*	*Genotype*	*%*	*n*	*Diplotype subgroup*	*Diplotype*	*%*	*n*
No variant allele	GG	38	16	CC	24	10	Wild-type	GG CC	21	9
1 variant allele	All	52	22	CT	50	21	Heterozygous[†]	All	67	28
	GT	95	21					GT CT	36	15
	GA	5	1					GT TT	14	6
								GG CT	12	5
								GA CC	2	1
								TA CT	2	1
2 variant alleles	All	10	4	TT	21	9	Homozygous variants	TT TT	7	3
	TT	75	3							
	TA	25	1							
Undetermined		0	0		5	2	Undetermined[‡]		5	2
Total		100	42		100	42	Total		100	42

[†]The 'heterozygous' group was defined as individuals being at least heterozygous for either 2677G>T/A or 3435C>T variants, or both. In our study population, five diplotypes were considered in this group.
[‡]For two individuals, the 3435C>T genotype could not be determined with the PCR method used in our laboratory. Therefore, the 2677–3435 diplotype was defined as 'undetermined' in these two patients.

36% of oseltamivir-treated patients, which is considerably higher than previously reported. In an observational retrospective study, the prevalence of NPAE reached 3.84% [7]. The incidence of postmarketing reports of NPAE in the Roche global safety database among Japanese and American children reached 99 and 19 per million, respectively. However, in a prospective study specifically designed to assess oseltamivir-related NPAE (hallucinations, delirious speech, frightening episodes, abrupt anger, abnormal activities leading to accidents, and putting anything unusual into the mouth) in Japanese children, the incidence reached 13% [21]. The higher incidence of NPAE observed in our cohort may be related to the fact that sleeping disorders were not included in the previous studies. When excluding sleeping disorders, the incidence of NPAE in our cohort decreased to 21%. Moreover, we actively sought NPAE whereas the majority of studies were based on spontaneous reports; it is therefore possible that only serious and/or major NPAE were reported by others. It is also likely that some of the reported NPAE were owing to the disease itself or other comorbidities. The H1N1/09 virus has a stronger neurotropism than other influenza viruses, thereby explaining the lower incidence of NPAE reported in previous studies [22,23]. Moreover, some of these studies were not limited to pediatric patients. As oseltamivir and/or H1N1 may be more neurotropic in children than in adults, this could contribute to the higher incidence of NPAE in our cohort [7].

As none of our patients stopped oseltamivir treatment because of NPAE and all patients with NPAE completely recovered at the end of the treatment, we can assume that their clinical significance is possibly low. However, as there is actually few data concerning oseltamivir treatment in children, we think it is important to report it, until larger studies prove the safety of this drug.

Whether or not oseltamivir could be incriminated in more severe NPAE should be more extensively evaluated. In an industry-sponsored review, Toovey *et al.* demonstrated that there was no clinical or pharmacological evidence to incriminate oseltamivir, or its metabolite, for an increased risk of NPAE [7]. However, in a recent Japanese prospective study with more than 10,000 seasonal influenza-infected patients, the authors demonstrated a higher risk of NPAE in those treated with oseltamivir [21].

One cannot exclude that the potentialization of two variants, one on 3435C>T and one on 2677G>T/A, could alter the expression and/or function of *ABCB1*, and thereby increase cerebral oseltamivir concentration and adverse CNS events. The molecular mechanisms explaining alteration of P-gp phenotype by *ABCB1* 3435 and

Table 3. Characteristics of neuropsychiatric adverse events in the 15 patients who presented ≥1 neuropsychiatric adverse event.

Type of NPAE	%	n
Nightmare	40	6
Agitation	40	6
Irritability	33	5
Sleeping disorder	7	1
Seizure	7	1

NPAE: Neuropsychiatric adverse events.

Table 4. Frequency of neuropsychiatric adverse events according to the presence of variant alleles.

Variant alleles	2677G>T/A		3435C>T	
	%	n	%	n
No variant alleles	31	5	20	2
1 variant allele	36	8	48	10
2 variant alleles	50	2	22	2
Undetermined	0	0	NA	1

NA: Not applicable.

2677 variants, alone or in combination, remain controversial. Among the possible hypothesis, modifications of mRNA folding and stability as well as potential strong linkage disequilibrium with a functional distinct allele have been proposed [24,25]. As linkage disequilibrium exists between SNP in exon 26 (3435C>T) and exon 21 (2677G>T/A), several genotype combinations can be described. Three common genotype combinations are found in more than 70% of the individuals reported in most studies, 2677GT–3435CT, 2677GG–3435CC and 2677TT–3435TT [20]. While comparing NPAE frequency according to the paired genotypes in our study population (2677GG–3435CC [wild-type homozygous] vs 2677TT–3435TT [homozygous variants]), we observed that the frequency of NPAE displayed a 'genotype-trend effect' with the variant and the wild-type subgroups at the two far ends. Wild-type homozygous presented much less NPAE compared with variant homozygous (11 vs 67%), while heterozygous individuals for at least one variant were between wild-type and homozygous variants (39%). However, the small number of patients enrolled in our study is a major limitation of our study and could explain the lack of statistically significant difference. Another limitation could be the high proportion of at-risk patients in our cohort, which might not represent typical flu patients. However, these patients represent the population who will mainly benefit from antiviral treatment. Thus, we think that our results could certainly be extrapolated to other settings. Although careful conclusions should be drawn owing to the small sample size of our study and the lack of control group, our results might suggest that variant homozygous individuals could be more vulnerable to NPAE, maybe as a consequence of a greater penetration of oseltamivir in the CNS. These observations can be considered to provide a signal deserving further confirmation by independent investigations. The association between the development of NPAE and *MDR1* polymorphisms has been demonstrated for the antimalarial drug mefloquine by Aarnoudse *et al.* [26]. The above mentioned study identified the 1236C>T, 2677G>T/A, and 3435C>T *MDR1* polymorphisms as risk factors for neuropsychiatric adverse effects in female mefloquine users. A haplotype-based analysis showed even more pronounced results, the homozygous 1236–2677–3435 TTT genotype being at higher risk. So far 48 SNP have been identified for the *ABCB1* gene [27]. Other variants not tested here could therefore also play a role. However, these other variants have been much less studied and their consequences on the pharmacokinetics of drug substrate and on protein expression/function are hypothetical.

We did not compare oseltamivir-treated and nontreated patients in terms of proportion of NPAE, which constitutes another limitation of our study. The lack of control group is owing to the fact that our study was an observational study with a primary objective to examine the safety profile of oseltamivir in children during the H1N1 pandemia. It is therefore impossible to distinguish the proportion of NPAE that could be related to H1N1 alone.

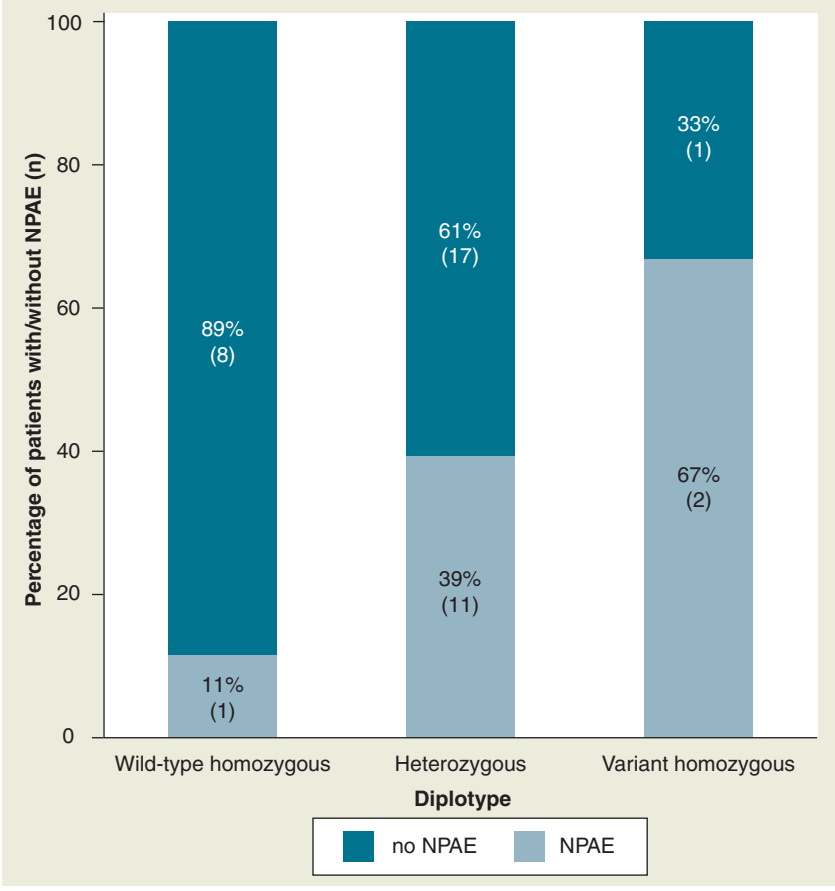

Figure 2. Frequency of neuropsychiatric adverse events according to the *ABCB1* 2677G>T/A-3435C>T diplotype.
NPAE: Neuropsychiatric adverse events.

Conclusion

Our study brings new insight concerning potential neurotoxicity of oseltamivir in children and adolescents. Our results show that NPAE are more frequent than expected when systematically assessed and suggest a possible influence of *ABCB1* polymorphisms in NPAE pathogenesis. Variants homozygous individuals (2677TT–3435TT) appeared to be more vulnerable to oseltamivir-induced NPAE, maybe as a result of an enhanced permeability of the blood–brain barrier to oseltamivir.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Oseltamivir is a prodrug that is extensively converted to the active metabolite oseltamivir carboxylate.
- Neuropsychiatric adverse events (NPAE) have been described in children taking oseltamivir, mainly in Japan.
- *In vitro* and *in vivo* studies on P-gp over-expressing cells and on mdr1a/1b knockout mice showed that oseltamivir, but not its metabolite, is a substrate of P-glycoprotein.

Subjects & methods

- During the H1N1 pandemia, we conducted a prospective cohort study to examine the safety profile of oseltamivir in children; to assess if the presence of *ABCB1* 3435C>T and 2677G>T/A variants could increase the risk of NPAE in oseltamivir-treated children.
- All adverse events were systematically recorded with a 30 days follow-up.

Results

- Among the 109 patients enrolled in the study, 75 patients had H1N1 disease confirmed by PCR, of which 54 were treated with oseltamivir, and of which 42 had a genetic analysis and responded to our 30-days follow-up questionnaire.
- A strong linkage disequilibrium was observed between 3435C>T and 2677G>T/A (D´ = 0.916).
- A total of 36% (15/42) of the patients presented NPAE, possibly related to oseltamivir, described as nightmares (6/15 patients), agitation (6/15), irritability (5/15), sleeping disorders (1/15) and seizures (1/15).
- We examined the association between the development of NPAE and 2677-3435 diplotype. A total of 11% (1/9) of the wild-type homozygous (2677GG-3435CC) presented a NPAE, compared with 39% (11/28) of the heterozygous and 67% (2/3) of the variant homozygous (2677TT-3435TT; p = 0.149, nonsignificant). The frequency of NPAE displayed a 'genotype-trend effect' with the variant and the wild-type subgroups at the two far ends.

Discussion & conclusion

- Our results bring new insight concerning potential neurotoxicity of oseltamivir in children and adolescents, suggesting that variant homozygous individuals could be more vunerable to NPAE, maybe as a consequence of a greater penetration of oseltamivir in the CNS.
- These observations can be considered to provide a signal deserving further confirmation by independent investigations.

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Part 4

Role of drug-drug interactions in drug safety

Introduction to the article

“Ing Lorenzini K, Girardin F. Direct-acting antiviral interactions with opioids, alcohol or illicit drugs of abuse in HCV-infected patients. Liver Int. 2020 Jan;40(1):32-44.”

This article provides an overview of drug-drug interactions (DDIs) between direct-acting antiviral (DAA) drugs used in the treatment of hepatitis C and commonly co-used substances, including opioids, alcohol, and drugs of abuse. Indeed, people with substance use disorders constitute the majority of hepatitis C cases in high-income regions. The risk of DDIs between DAAs and substances of abuse could be a barrier to DAAs access and successful treatment. We reviewed the available evidence regarding pharmacokinetic and pharmacodynamic DDIs between these two categories of drug/substance. All DAAs are P-gp substrates. Some of them are substrates of other transporters, and some of them are also CYP substrates. Therefore, any substance that is capable of inhibiting these transporters and/or enzymes may increase DAAs concentrations and risk of ADRs. Conversely, substance inducing these transporters and/or enzymes may decrease DAAs concentrations which can result in therapeutic failure. Given their high costs, inefficacy is unwanted. Based on their respective pharmacological properties and available studies, no clinically relevant DDI is expected with opioids and stimulants, including an increase in opioid toxicity through CYP/transporter inhibition by DAAs (e.g. methadone-induced QT prolongation). Although we were concerned about a potential decrease in DAAs concentrations in the case of chronic alcohol use through its ability to induce P-gp, such an interaction was not observed in large cohort studies. In conclusion, our article suggests that the interaction potential of DAAs with most opioids and illicit drugs is limited.

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REVIEW

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Direct-acting antiviral interactions with opioids, alcohol or illicit drugs of abuse in HCV-infected patients

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Abstract

The hepatitis C virus (HCV) prevalence is extremely high in patients who consume and inject illicit drugs. Concerns about poor adherence and fear of interaction with drugs of abuse could constitute further disincentive for treatment initiation in these patients. We discussed the pharmacokinetics (PKs) and pharmacodynamics (PD) of currently prescribed direct antiviral agents (NSA5 inhibitors: daclatasvir, elbasvir, ledipasvir, pibrentasvir, velpatasvir; NS5B inhibitor: sofosbuvir; NS3/4A protease inhibitors: glecaprevir, grazoprevir, voxilaprevir) and most common substances of abuse (opioids: buprenorphine, fentanyl, heroin, methadone, morphine, oxycodone; stimulants: amphetamines, cathinones, cocaine; cannabinoids; ethanol). Overall, most direct-acting antivirals (DAAs) are substrates and inhibitors of the transmembrane transporter P-glycoprotein (P-gp), and several of them are metabolized by cytochrome P450 enzymes. Clinically relevant interactions are associated with P-gp and CYP3A modulators. Most substances of abuse are eliminated by Phase I and Phase II metabolizing enzymes, but none of them are either major inhibitors or inducers. PK studies did not show any relevant interactions between DAA and methadone or buprenorphine. Based on pharmacological considerations, neither efficacy loss nor adverse drug event associated with detrimental interaction are expected with opioids, stimulants, cannabinoids and ethanol. In summary, our literature review shows that the interaction potential of DAA with most opioids and illicit drugs is limited and should not be a hurdle to the initiate DAA.

KEYWORDS
alcohol, direct-acting antivirals, drug-drug interaction, opioids, stimulants, substance abuse

1 | INTRODUCTION

Chronic viral hepatitis C infections are a major health problem, with approximately 1.75 million new cases worldwide (2015 estimation).[1,2] The hepatitis C virus (HCV) prevalence is variable across the world: around 0.1%-1.0% in European countries, 2.0%-6.5% in Central Asia and up to 7.0% in African countries.[3] In western countries, the major source of new HCV infections remains intravenous drug use, which

Abbreviations: 4-MMC, 4-methyl-N-methylcathinone; 6-AM, 6-acetylmorphine; Ab, antibody; ADH, alcohol dehydrogenase; AE, adverse event; AUC, area under the curve; AZCERT, Arizona Education and Research on Therapeutics; BBV, blood-borne viral; BCRP, breast cancer resistance protein; CBD, cannabidiol; C_{max}, maximal concentrations; CYP450, cytochrome P450; DAA, direct-acting antiviral; DCV, daclatasvir; EBR, elbasvir; GZR, grazoprevir; HBV, hepatitis B virus; hCE, human carboxylesterase; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IDU, injection drug use; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; MDMA, 3,4-methylenedioxymethamphetamine; non-IV, non-intravenous; NS, non-structural; OATP, organic-anion-transporting polypeptide; OR, odds ratio; OST, opioid substitution therapy; OUD, opioid use disorder; PD, pharmacodynamics; P-gp, P-glycoprotein; PK, pharmacokinetic; PWID, people who inject drugs; PWSUD, people with substance use disorder; QALY, quality-adjusted life-year; SVR, sustained virologic response; TdP, torsades de pointes; UGT, uridine glucuronosyltransferase; Δ9-THC, delta-9-tetrahydrocannabinol.

caused 390 000 new cases in 2015.[2] People with substance use disorders (PWSUDs) constitute the majority of incident (75%) and prevalent (80%) HCV cases in high-income regions.[4] Thus, people who inject drugs (PWID) were more frequently HCV antibody (Ab)-positive than non-intravenous (non-IV) drug users.[5]

Since 2014, the development of direct-acting antivirals (DAAs) revolutionized the management of chronic HCV infections, with higher rates of sustained virologic response (SVR) (>90%) and shortened treatment duration (8 to 12 weeks). Current available regimens consist of pangenotypic fixed-drug combinations (sofosbuvir/velpatasvir or glecaprevir/pibrentasvir), or of alternative genotype-specific regimen that includes sofosbuvir, ledipasvir, daclatasvir, grazoprevir/elbasvir and tritherapy with the fixed-dose association of sofosbuvir/velpatasvir/voxilaprevir.[6,7]

In PWSUD, the eradication of HCV could decrease the virus circulation community, reducing the infection rate.[8] However, several barriers to HCV therapy have been identified, such as high rate of psychiatric disorders (psychosis and depression),[9] poor adherence, ongoing substance use including alcohol use, lower responses to therapy, medication price and the risk of reinfection.[10] The risk of interactions of DAAs with drugs used in substance disorders, such as opioid substitution therapy (OST), as well as with illicit or recreational substances (cocaine, alcohol), can constitute a barrier to DAA access in PWSUD.

DAAs could be subject to drug-drug interactions (DDI), as they are transformed by metabolic enzymes and substrates of efflux transporters. They can also act as perpetrator of DDI if they modulate enzyme or transporter activity.[7] The evidence regarding relevant DDI with most of the potential concomitantly prescribed drugs has been recently reviewed.[7,11]

Key points

- Overall, the interaction potential of direct-acting antivirals (DAAs) with most opioids and illicit drugs is limited.
- Most DAA inhibits P-gp, and to a lesser extent CYP3A.
- Interactions are theoretically possible with opioids including methadone, but not confirmed in pharmacokinetic studies.
- Given the frequent association with methadone, there is no evidence of an increased risk of long QT or torsade-de-pointes.

2 | METHODS

In order to review the main pharmacokinetic (PK) and pharmacodynamic (PD) characteristics and interaction potential of currently available DAAs (based on 2018 EASL recommendations[6]), and of selected substances of abuse, we performed a PubMed search of articles published until June 2019. The following keywords were used: drug-drug interaction, pharmacokinetics, pharmacodynamics, daclatasvir, elbasvir/grazoprevir, glecaprevir/pibrentasvir, sofosbuvir/ledipasvir, sofosbuvir/velpatasvir, sofosbuvir/velpatasvir/voxilaprevir, substance of abuse, opioids, buprenorphine, fentanyl, methadone, morphine, oxycodone, amphetamine, ecstasy, cathinones, cocaine, cannabis and ethanol. The paritaprevir/ombitasvir/ritonavir ± dasabuvir combination was not considered in our review since this regimen is no longer recommended.[11,12]

The following article types were eligible: PK/PD reviews, original articles, articles on physiologically based pharmacokinetic (PBPK) models and case reports. Only articles written in English or in French were selected. The following types of studies were eligible: in vitro PK studies, human clinical studies (PK phase 1 studies or phase 2/3 clinical studies) and post hoc or pooled analysis of clinical studies. Only studies regarding interactions of currently available DAAs with the selected substances of abuse were eligible.

For currently available DAAs, we included in our search the summary of product characteristics and the European public assessment report on the European Medicines Agency website (https://www.ema.europa.eu/en).

Finally, we performed a Google search to find congress abstracts or conference proceeding reporting unpublished interaction studies or data.

Papers involving other molecules than the keywords, and not considering PK, PD or drug-interaction data, were excluded.

3 | CLINICAL PHARMACOLOGY OF DAA

The currently available drugs in Europe are presented in Table 1, and their main PK and PD characteristics in Table 2, and in Figures 1 and 2.

3.1 | Daclatasvir

Daclatasvir (DCV) is an inhibitor of the HCV non-structural 5A (NS5A) protein approved for HCV genotypes 1, 3 and 4 in association with sofosbuvir, with or without ribavirin.[13] It is readily absorbed after oral administration, with maximal concentrations (C_{max}) achieved after 1-2 hours, and an absolute bioavailability of 67%, and is highly bound to plasma proteins (99%). DCV is a substrate of the P-glycoprotein (P-gp) efflux transporter, and is metabolized by cytochrome P450 (CYP) 3A isoenzymes, predominantly CYP3A4. A majority of the dose (88%) is excreted in the faeces and 7% in urine. The mean terminal half-life after multiple dosing is 12-15 hours. DCV is an inhibitor of P-gp, organic-anion-transporting polypeptide (OATP) 1B1 and breast cancer resistance protein (BCRP) transporters.[14] Interactions may occur when DCV is used concomitantly with CYP3A and P-gp inhibitor or inducers, and with P-gp, OATP and BCRP substrates.

From a safety point of view, the most common adverse events (AEs) in studies combining DCV and sofosbuvir were headache,

nausea, diarrhoea, arthralgia and cough.[14] Regarding cardiac safety, a thorough QT study with therapeutic (60 mg) and supratherapeutic doses (180 mg) showed the absence of QTc variations or related repolarization abnormalities.[15]

3.2 | Elbasvir/grazoprevir

Elbasvir (EBR) and grazoprevir (GZR) are available as a 50/100 mg fixed-dose combination, approved for HCV genotypes 1a, 1b and 4.[16] EBR is an inhibitor of the HCV NS5A while GZR inhibits NS3/4A protease. EBR and GZR's bioavailability is 20% to 40%, and C_{max} is achieved after 2-4 hours.[17,18] Both compounds display extensive protein binding (free fraction: 1%).[18] Excretion into faeces as parent drugs accounts for 75% to 80% of elimination of both EBR and GZR, the remaining 20% being excreted as oxidative metabolites formed via CYP3A.[17] Both compounds are P-gp substrates. In addition, GZR (but not EBR) is a substrate of OATP1B1 and OATP1B3.[17,18] Their steady-state elimination half-lives in HCV-infected subjects were 24 and 31 hours for EBR and GZR respectively.[18] Neither EBR nor GZR are potent inhibitors or inducers of CYP or uridine glucuronosyltransferase (UGT) enzymes in vitro,[18] but GZR is a weak inhibitor of CYP3A. EBR is a mild inhibitor of intestinal P-gp and BCRP, and GZR is an intestinal BCRP inhibitor.[17] Clinically relevant DDI are likely to occur with moderate and strong CYP3A and P-gp inducers (but not with CYP3A inhibitors), and when GZR is combined with OATP1B inhibitors.[17] CYP3A and P-gp/BCRP substrates with a narrow therapeutic range may be subject to DDI with EBR/GZR (increases in plasma concentrations).

The most frequently reported AEs were fatigue (17.0%), headache (16.4%), nausea and dose-dependent increases in liver function markers.[18,19]

3.3 | Glecaprevir/pibrentasvir

Glecaprevir and pibrentasvir are available as a 100/40 mg fixed-dose combination, approved for all HCV genotypes.[19] The recommended dose is 300/120 mg once daily (three tablets) to be taken with a meal. Glecaprevir is HCV NS3/4A protease inhibitor while pibrentasvir inhibits HCV NS5A.[20] Their C_{max} is attained after 5 hours. Both drugs are extensively bound to plasma proteins (>95%). Glecaprevir undergoes limited CYP3A-mediated metabolism,[20] with 26% of the dose excreted as oxidative metabolites,[19] whereas pibrentasvir is not metabolized.[20] Both drugs are excreted in the faeces. Their elimination half-lives are 6-9 and 23-29 hours respectively.[20] Glecaprevir is a substrate and inhibitor of P-gp, BCRP and OATP1B1/B3. Pibrentasvir is a substrate of P-gp and of BCRP,[21] and inhibits P-gp, BCRP and OATP1B1/B3.[19] Clinical DDI studies have confirmed clinically relevant inhibition of P-gp, BCRP and OATP1B1/B3.[22] Finally, glecaprevir and pibrentasvir are weak inhibitors of CYP3A and UGT 1A1 in vitro and in vivo.[19,21] Therefore, CYP3A, UGT1A1, P-gp/BCRP and OATP1B substrates with a narrow therapeutic range are subject to DDI with glecaprevir and pibrentasvir.

The most frequently reported AEs were headache (13%), fatigue (11%) and nausea (8%).[20]

3.4 | Sofosbuvir/ledipasvir

The fixed-dose combination of sofosbuvir and ledipasvir contains 400 and 90 mg of each active substance respectively. It is approved for the treatment of HCV genotypes 1, 4, 5 and 6.[23] Sofosbuvir is a prodrug that requires several steps bioactivation to GS-461203, the pharmacologically active nucleoside analog triphosphate metabolite, which inhibits NS5B,[24,25] and is ultimately dephosphorylated to an inactive metabolite, GS-331007.[24,25] Ledipasvir inhibits NS5A. Sofosbuvir and ledipasvir C_{max} are reached after 1 and 4 hours respectively. Their protein bound fractions are, respectively, 65% and 99%.[24] Sofosbuvir and its metabolites are not substrates of CYP or UGT enzymes. Ledipasvir and sofosbuvir itself are substrates for P-gp and BCRP, but not GS-331007.[24,26] Sofosbuvir elimination is essentially non-renal, whereas renal clearance is the major elimination pathway for GS-331007.[25] The latter has a half-life of 27 hours.[24] Ledipasvir half-life is 50 hours and it is mainly excreted in faeces (>70%).[27] Sofosbuvir and GS-331007 do not display significant inhibition or induction of CYP, UGT1A1 and main drug transporters.[25] Ledipasvir does not inhibit major human CYP,[27] but has shown to inhibit the transporters P-gp and BCRP

TABLE 1 Available drugs and fixed-dose combinations according to the EASL 2018 recommendations[6]

Drug or fixed-dose combinations	Brand name	Recommended dose	Comment
Daclatasvir	Daklinza®	60 mg qd	In combination with other drugs
Elbasvir/grazoprevir	Zepatier®	50/100 mg qd	
Glecaprevir/pibrentasvir	Maviret®	100/40 mg qd	
Sofosbuvir	Sovaldi®	400 mg qd	In combination with other drugs
Sofosbuvir/ledipasvir	Harvoni®	400/90 mg qd	
Sofosbuvir/velpatasvir	Epclusa®	400/100 mg qd	
Sofosbuvir/velpatasvir/voxilaprevir	Vosevi®	400/100/100 mg qd	

Note: bid: twice daily; qd: once daily.

TABLE 2 Main PK and PD characteristics of DAA

Drug	Metabolism and transport	Elimination	CYP/transporter inhibition or induction	Main adverse effects	References
NS5A inhibitors					
Daclatasvir	CYP3A, P-gp	Faeces	P-gp, OATP1B1, BCRP inhibition	Headache, nausea, diarrhoea, arthralgia and cough	14
Elbasvir	CYP3A, P-gp	Faeces	P-gp, BCRP inhibition	Fatigue, headache, nausea	17,18
Ledipasvir	P-gp, BCRP	Faeces	P-gp, BCRP inhibition	Headache, fatigue, insomnia, nausea, diarrhoea	23,24
Pibrentasvir	No metabolism, P-gp (BCRP)	Faeces	CYP3A, UGT1A1, P-gp, BCRP, OATP1B1 and OATP1B3 inhibition	Headache, fatigue, nausea	19,20
Velpatasvir	Minor metabolism by CYP2B6, CYP2C8 and CYP3A4, P-gp, BCRP	Faeces	P-gp, BCRP, OATP1B1, OATP1B3, OATP2B1 inhibition	Headache, fatigue	29,30
NS5B inhibitors					
Sofosbuvir	P-gp, BCRP	Urine (GS-331007)	—	Headache, fatigue, insomnia, nausea, diarrhoea	23,24
Protease inhibitors					
Glecaprevir	CYP3A, P-gp, BCRP, OATP1B1 and OATP1B3	Faeces	CYP3A, UGT1A1, P-gp, BCRP, OATP1B1 and OATP1B3 inhibition	Headache, fatigue, nausea	19,20
Grazoprevir	CYP3A, P-gp, OATP1B1 and OATP1B3	Faeces	CYP3A, BCRP inhibition	Fatigue, headache, nausea	17,18
Voxilaprevir	CYP3A, P-gp, BCRP, OATP1B1 and OATP1B3	Faeces	P-gp, OATP1B1 and OATP1B3 inhibition	Headache, fatigue, diarrhoea, nausea	33,34

Abbreviations: BCRP, breast cancer resistance protein; CYP, cytochrome; DAA, direct-acting antiviral; PD, pharmacodynamic; Pk, pharmacokinetic.

in vitro.[23,26] The concomitant use of P-gp and/or BCRP inducers may result in virologic failure. P-gp and/or BCRP substrates with a narrow therapeutic range may see their exposure increase in the presence of sofosbuvir/ledipasvir.

Patients treated with the association may experience headaches, fatigue, insomnia, nausea and diarrhoea.[24] In healthy volunteers, the corrected QT interval was not prolonged after single therapeutic (400 mg) and after supratherapeutic doses of sofosbuvir (1200 mg) and ledipasvir 120 mg (twice daily) in thorough QT studies.[25,28]

3.5 | Sofosbuvir/velpatasvir

The pangenotypic combination of sofosbuvir and velpatasvir contains 400 and 100 mg of each active substance respectively.[29] Velpatasvir is an HCV NS5A protein inhibitor. Its C_{max} is reached 3 hours after oral administration. Velpatasvir is extensively bound to plasma proteins (>99%), and undergoes minor metabolism by CYP2B6, CYP2C8 and CYP3A4, and is excreted (77% as parent drug) in the faeces with a median terminal plasma half-life of 15 hours. Velpatasvir is transported by P-gp and BCRP, and is also an inhibitor of P-gp, BCRP, OATP1B1/B3 and OATP2B1.[30,31] There is a risk of therapeutic failure when the association is administered with P-gp and/or BCRP inducers. Substrates of P-gp, BCRP and OATP can be subject to an increase in their exposure when administered with sofosbuvir/velpatasvir.[30]

The most common AEs when sofosbuvir/velpatasvir is used without ribavirin are headache and fatigue.[30,31] In healthy volunteers, the corrected QT interval was not significantly prolonged by therapeutic sofosbuvir (400 mg) or supratherapeutic doses of velpatasvir (500 mg) or sofosbuvir (1200 mg).[30]

3.6 | Sofosbuvir/velpatasvir/voxilaprevir

The pangenotypic combination of sofosbuvir, velpatasvir and voxilaprevir contains 400, 100 and 100 mg of each active substance.[32] Voxilaprevir is a reversible NS3/4A protease inhibitor. It reaches C_{max} 4 hours after administration. Food substantially increases the systemic exposure. It is extensively bound to plasma proteins (>99%) and undergoes metabolism by CYP3A4. Voxilaprevir is eliminated through biliary excretion, 40% as the parent drug, with a half-life of 33 hours.[11,33] Voxilaprevir is a substrate of P-gp, BCRP and OATP1B1/B3.[34] Inhibition of OATP1B1/B3 at clinically achieved concentrations is reported.[34] There is a risk of therapeutic failure when inducers of CYP3A, P-gp and/or BCRP are co-administered. Concentrations of OATP1B1/B3 substrates can increase when administered with sofosbuvir/velpatasvir/voxilaprevir.[33]

The most frequently reported AEs with this association are headache, fatigue, diarrhoea and nausea. Voxilaprevir was not shown to significantly prolong the QTc interval when given at nine times the recommended dose.[33]

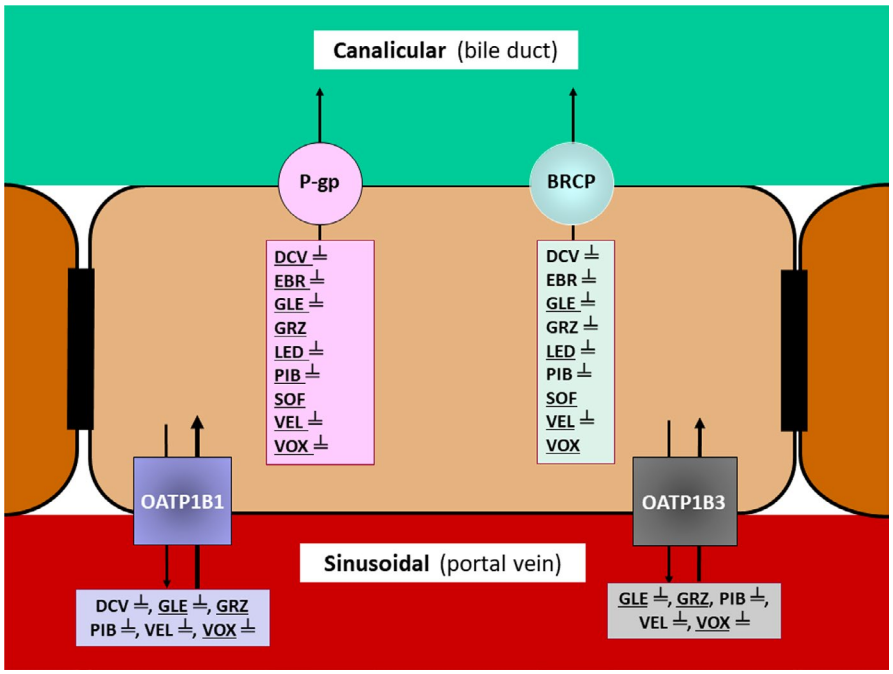

FIGURE 1 Transmembrane transport of direct-acting antiviral (substrates are underlined) and inhibition effect (⊥) (alphabetic order). BCRP, breast cancer resistance protein; DCV, daclatasvir; EBR, elbasvir; GLE, glecaprevir; GRZ, grazoprevir; LED, ledipasvir; OATP, organic-anion-transporting polypeptide; P-gp, P-glycoprotein; PIB, pibrentasvir; SOF, sofosbuvir; VEL, velpatasvir; VOX, voxilaprevir

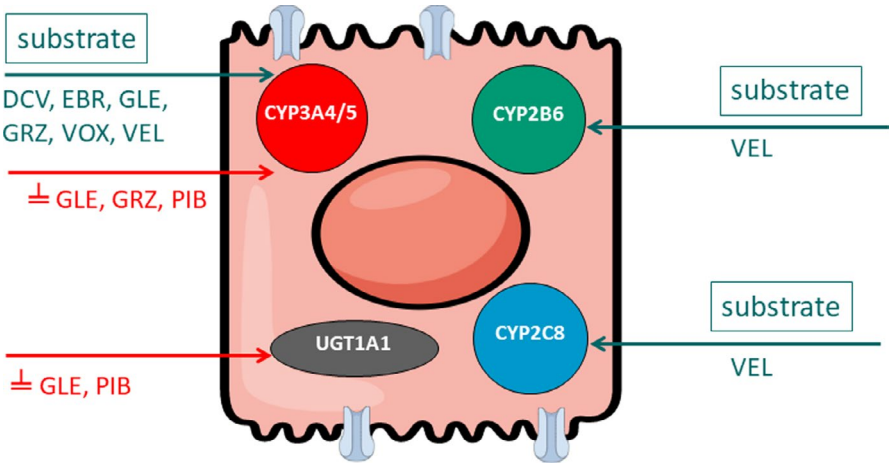

FIGURE 2 Metabolism of direct-acting antiviral and inhibitor (⊥) (alphabetic order). DCV, daclatasvir; EBR, elbasvir; GLE, glecaprevir; GRZ, grazoprevir; PIB, pibrentasvir; VEL, velpatasvir; VOX, voxilaprevir

4 | CLINICAL PHARMACOLOGY OF SUBSTANCES OF ABUSE

The main PK/PD characteristics of selected substances of abuse are presented in Table 3.

4.1 | Opioids

People often start with oral non-medical use of opioids, and move to more efficient routes of administration, such as insufflation, smoking or injection, and possibly initiate heroin use.[35] Data on Drug Abuse Trends showed a first increase in the misuse of opioids between 2004 and 2011.[36] Synthetic opioids, such as fentanyl, are major contributors to opioid-related overdoses.[37] The management of opioid use disorder (OUD) requires an integrated treatment that includes opioid substitution therapy (OST). Methadone was the first medication approved in this indication, and buprenorphine is also approved. Other available options include intravenous diamorphine (medical heroin), levomethadone and slow-release oral morphine.[38]

All opioids are metabolized through two major enzyme systems, CYP450 and UGT, but few of them are inhibitors or inducers of metabolizing enzymes or transporters; only methadone is identified as a CYP2D6[39] and P-gp inhibitor.[40] Excepted methadone, opioids are not expected to be significant perpetrator of CYP- or P-gp-mediated DDI with DAA.

Buprenorphine is a semisynthetic derivative of thebaine with partial opioid agonist properties, metabolized to norbuprenorphine by CYP3A (65%) and to a lesser extent by CYP2C8. Buprenorphine and norbuprenorphine undergo extensive Phase II metabolism by UGT, mainly UGT2B7 (>40%), followed by UGT1A1 and UGT1A3.[41]

TABLE 3 Main PK and PD characteristics of substances of abuse

Drug	Metabolism and transport	Elimination	CYP/transporter inhibition or induction	Main adverse effects	References
Opioids					
Buprenorphine	CYP3A, CYP2C8, UGT2B7, UGT1A1, UGT1A3	Faeces	—	Tolerance, dependence, cognitive effects, sedation, delirium, constipation, vertigo, nausea, respiratory depression	41,45 46,47
Fentanyl	CYP3A, P-gp	Urine	—	As for buprenorphine	41,43 46,47
Heroin	hCE then as morphine		—	As for buprenorphine	43 46,47
Methadone	CYP2B6, P-gp	Faeces and urine	CYP2D6, P-gp inhibition	As for buprenorphine, and QT prolongation, TdP	39,41,44,45 46,47
Morphine	UGT2B7, UGT1A1, UGT1A3, UGT1A9, CYP3A, CYP2C8, P-gp	Urine	—	As for buprenorphine	41,43,45,106 46,47
Oxycodone	CYP3A, CYP2D6	Urine	—	As for buprenorphine	41,45 46,47
Stimulants					
Amphetamine and methamphetamine	CYP2D6, CYP2C	Urine	—	Anorexia, insomnia, nausea, vomiting, increases in blood pressure and heart rate	49,52,53
MDMA	CYP2D6 CYP1A2, CYP2B6, CYP3A	Urine	CYP2D6 inhibition	Perceptual disturbances, increases in blood pressure and heart rate, mydriasis, derealization, panic attacks, delirium	54
Cathinones	CYP2D6	Urine	CYP2D6 inhibition	Tachycardia, hypertension, agitation, hallucinations confusion, creatine kinase elevation	50,56,57
Cocaine	hCE	Urine	—		40,59
Various					
Cannabinoids	CYP2C9, CYP3A4	Faeces (65%-80%) and urine (20%-35%)	CYP450, P-gp inhibition (probably minor)	Asthenia, balance problems, confusion, dizziness, dry mouth, fatigue, hallucinations, nausea, vomiting, drowsiness	40,64,66-68
Ethanol	ADH, CYP2E1, CYP2A1, CYP3A4	Urine, sweat, saliva, tears, expired air	CYP450, P-gp induction?	Depressive effect on the central nervous system: anxiolytic effect, disinhibition of behaviour, sedation, respiratory depression	73-75

Abbreviations: CYP, cytochrome; PD, pharmacodynamics; Pk, pharmacokinetic.

Fentanyl is a synthetic opioid agonist, 50 times more potent than morphine, metabolized to norfentanyl by CYP3A and transported by P-gp.[41]

Heroin is rapidly metabolized by a sequential hydrolysis/deacetylation to 6-acetylmorphine (6-AM) and morphine.[42] The enzymatic metabolism is mediated mainly by human carboxylesterase 1 (hCE) and in part by hCE-2. Heroin has a very low affinity for μ-opioid receptors, and it acts as a highly lipophilic prodrug of its active metabolites 6-AM, morphine and morphine-6-glucuronide (M6G).[43]

Methadone is a synthetic opioid receptor agonist generally used as the racemic mixture of (R)- and (S)-methadone.[41] It is extensively metabolized by CYP450 enzymes, CYP2B6 being currently recognized as the major isoform in human.[44] Methadone is a P-gp substrate.[41]

Morphine is conjugated mainly by UGT2B7 to the inactive metabolite morphine-3-glucuronide (M3G) and, to a lesser extent, to the pharmacological active compound M6G.[41] UGT1A1, 1A3 and 1A9 are also involved but to a lesser extent.[43]

Oxycodone is a semisynthetic opioid that is mainly (80%) metabolized by CYP3A to noroxycodone, and to a lesser extent (10%) by CYP2D6 to oxymorphone, which is pharmacologically active.[41,45]

All opioids share a common profile of potential AEs that include among others tolerance and dependence, cognitive effects, sedation, delirium, constipation, vertigo, nausea and respiratory depression.[46,47] Reported AE also include cardiovascular effects, the most common being the prolongation of the QT interval, which can lead to torsades de pointes (TdP) and sudden death. The arrhythmogenicity

of the main available opioids has been recently reviewed.[48] The website https://crediblemeds.org/, created and maintained by Arizona Education and Research on Therapeutics (AZCERT), is also a recommended source of information for drug-induced QT prolongation. This website defines three main categories of risk: known, possible and conditional risk.

Methadone is classified within the known risk category, buprenorphine within the possible risk category and fentanyl, morphine and oxycodone are not classified in any category. There have been many reports and studies showing the potential of methadone to induce QT interval prolongation and TdP even in low doses.[48] Buprenorphine at conventional doses, by itself, does not appear to produce clinically significant QT interval prolongation or polymorphic ventricular arrhythmia.[48]

4.2 | Stimulants

4.2.1 | Amphetamine and derivatives

Amphetamine and its derivatives, which include 3,4-methylenedioxymethamphetamine (MDMA) or ecstasy, belong to the class of β-phenylethylamines and show chemical similarity with the catecholamine neurotransmitters, noradrenaline and dopamine.[49,50] After marijuana, these stimulants are the second most widely used group of illicit drugs worldwide. HCV infection is frequent among methamphetamine (N-methylated derivative of amphetamine) users as a consequence of unsafe injection methods, and as its use contributes to high-risk behaviours. One study in Veterans Affairs showed that 37% of HCV patients had a history of methamphetamine use. These patients were particularly prone to polysubstance use, alcohol and marijuana in particular.[51]

Amphetamine is metabolized to 4-hydroxyamphetamine via CYP2D6, while isoenzymes of the CYP2C subfamily mediate its deamination pathway. Amphetamine does not exert significant inhibition towards main CYP enzymes or P-gp.[52] Methamphetamine metabolism is also mediated by CYP2D6.[53] Amphetamine and methamphetamine are excreted through the kidneys.[52,53] MDMA metabolism occurs through two metabolic pathways, O-demethylation followed by catechol-O-methyltransferase (COMT)-catalysed methylation and/or glucuronide/sulphate conjugation; and N-dealkylation, deamination and oxidation to the corresponding benzoic acid derivatives conjugated with glycine. The involved enzymes are CYP2D6 and CYP1A2, and to a lesser extent CYP2B6 and CYP3A4. Moreover, MDMA is also a quasi-irreversible inhibitor of CYP2D6, through the formation of a metabolite-intermediate complex.[54]

As stimulants, amphetamines and derivatives can cause increases in blood pressure and heart rate, gastrointestinal symptoms such as nausea, vomiting and abdominal cramps.[49] After high dose and frequent methamphetamine use, psychotic episodes and neurotoxic effects such as memory deficits and impaired psychomotor can occur.[53] MDMA can produce panic attacks, delirium and brief psychotic episodes that usually resolve rapidly when the drug action wears off.[54]

4.2.2 | Cathinones

Synthetic cathinones are derivatives of the parent compound cathinone, a naturally occurring psychostimulant found in the khat plant, *Catha edulis*.[55] The most common AEs associated with the use of cathinones include tachycardia, hypertension, anxiety/agitation, hallucinations/delusions, confusion and creatine kinase elevation.[56]

In vitro studies have shown that cathinones are mainly metabolized by CYP2D6, but the involvement of other enzymes is possible.[50] For example, mephedrone is mainly metabolized by CYP2D6.[57] A study in healthy users of khat showed that the use of this plant resulted in a CYP2D6 inhibition and a marginal effect on CYP3A4 and CYP2C19 activities, owing to competitive inhibition by cathinone.[58] Potential interactions between cathinones and DAA are limited.

4.2.3 | Cocaine

Cocaine, the main alkaloid of *Erythroxylum coca*, is a powerful stimulant whose metabolism is mainly mediated by three esterases, pseudocholinesterase, human carboxylesterase-1 (hCE-1) and 2 (hCE-2). HCE-1 mediates the formation of benzoylecgonine, the main metabolite excreted in the urine. Pseudocholinesterase and hCE-2 catalyses the formation of ecgonine methyl ester.[59] Approximately, 85% to 90% of a dose are excreted in the urine, including 1% to 5% in unchanged form, and 75% to 90% of a dose as the metabolites benzoylecgonine and ecgonine methyl ester. Cocaine also undergoes oxidative metabolism by N-demethylation to pharmacologically active norcocaine. This metabolism is catalysed either by CYP3A4 or by a route involving both CYP and flavin-monooxygenases (2-step metabolism in the latter case).[59] Oxidative metabolism to norcocaine represents less than 10% of the biotransformation of cocaine.[60] Cocaine did not inhibit P-gp and BCRP in vitro.[40] The risk of interaction with DAA is, if any, very limited.

Cocaine blocks the presynaptic reuptake of norepinephrine and dopamine and acts as a powerful sympathomimetic agent. It has been associated with a variety of cardiac and other systemic complications. On the central nervous system, cocaine can cause cerebrovascular, neurological and psychological effects that include intracranial haemorrhage, seizures, movement disorders and psychiatric illness (such as psychosis, depression, decreased appetite).[61] Among the many complications exhibited by cocaine use, cardiovascular toxicities are very prominent and comprise hypertension, coronary spasm, arrhythmias, myocardial infarction, cardiomyopathy, atherosclerosis and coronary artery disease.[62] Pulmonary, hepatic and renal toxicities have also been reported.[61]

4.3 | Various

4.3.1 | Cannabinoids

The cannabis plant (*Cannabis sativa*) contains more than 100 different cannabinoids. Among them, delta-9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) are quantitatively important and of

medical interest.[63] Commonly observed AEs with cannabinoids included: asthenia, balance problems, confusion, dizziness, dry mouth, fatigue, hallucinations, nausea, vomiting and drowsiness.[64]

THC is essentially stored in adipose tissue and is slowly released into the bloodstream, with a long terminal half-life of 25-36 hours.[65] THC metabolism is catalysed by CYP2C9 and CYP3A4. CBD is metabolized by CYP3A4.[66] At high concentrations, THC and CBD have demonstrated a potential inhibitory effect on CYP450 in vitro.[67-72] Some data suggested that cannabinoids might inhibit P-gp but at high concentrations that are probably not achieved in vivo.[40] Potential interactions with DAA have not been studied but probably appear limited without loss of DAA activity.

4.3.2 | Ethanol

The vast majority of ethanol (>90%) is metabolized by liver alcohol dehydrogenase (ADH), whereas a small fraction (<6%) occurs via CYP2E1. These enzymes are inducible. Even though they account for a small percentage of ethanol metabolism, induction of CYP activity can increase ethanol elimination by more than 25%.[73]

The impact of ethanol on drug-metabolizing enzymes seems to differ after acute and chronic ingestion. Several in vitro studies have shown that some components of red wine inhibited CYP3A, and eventually CYP2C19 at high concentrations.[74] However, a study in healthy volunteers suggested the opposite. Indeed, acute red wine led to a 30%-40% decrease in the exposure of ciclosporine, a CYP3A substrate. The authors suggested that the mechanism could be decrease in ciclosporine solubility and absorption.[74] Consistent with in vitro data, a study in healthy volunteers showed that acute ethanol ingestion resulted in an average 30% increase in the area under the curve (AUC) of diazepam, a CYP2C19 (major pathway) and CYP3A substrate, although this observation was not confirmed by ulterior studies. Other clinical studies have shown the lack of significant impact of acute alcohol ingestion on the PKs of CYP3A substrates (triazolam, zolpidem, felodipine, verapamil, maraviroc, vardenafil).[75]

In an in vitro study, assessing the effects of chronic alcohol exposure on the expression of drug-metabolizing enzymes and drug transporters, ethanol strongly increased the mRNA expression of CYP2C19, CYP2E1 and ABCB1 after 1 and 3 weeks of exposure. Regarding ABCB1, this induction did not translate into an increase in efflux activity.[76] In another in vitro study, ethanol was shown to induce CYP3A4.[77] A study examining the liver biopsy from 12 patients with a history of excessive chronic alcohol consumption reported higher levels of CYP3A as compared to five patients with non-alcoholic hepatitis.[78] In a clinical study involving 20 individuals with moderate chronic alcohol consumption (average 2-3 drinks per day), the disposition of intravenous midazolam, a CYP3A substrate, was not altered as compared to 20 individuals without alcohol consumption. However, the oral availability of midazolam was reduced by 26% in the alcohol group, suggesting CYP3A induction at the small bowel level.[79] In another clinical study evaluating the PK of diazepam (CYP2C19 and CYP3A substrate), the AUC was lower in chronic alcohol drinkers than in healthy subjects, also suggesting an induced diazepam metabolism.[75] In conclusion, a modest induction of CYP3A could be expected in chronic alcohol users, but the change in drug PK may be confounded by the alteration of CYP enzyme activities as a result of chronic liver disease or mild cirrhosis rather than the presence of ethanol in the blood alone.[75]

Alcohol exhibits a dose-dependent effect on the central nervous system that can include disinhibition, sedation and respiratory depression. The cardiovascular effect can manifest as coronary vasodilatation after acute consumption, whereas chronic use can lead to increased blood pressure as well as arrhythmias and cardiomyopathy. Other effects associated with alcohol use are pancreatitis and liver diseases such as hepatitis and cirrhosis.[75]

5 | REVIEW OF CLINICAL EVIDENCES

5.1 | Interactions of DAA with opioids

The DDI between DCV and methadone, and the association buprenorphine-naloxone was assessed in a PK study including 25 subjects on stable OST (14 on methadone, 11 on buprenorphine/naloxone). This study showed no clinically relevant effect of DCV on methadone PK, whereas a raise in buprenorphine and norbuprenorphine exposure was observed (buprenorphine: AUC and C_{max} increased by 30%-40%). However, these increases were not considered to be clinically significant by the authors. None of the opioid had an impact on DCV PK as compared to historical data in healthy volunteers.[80]

The DDI between elbasvir/grazoprevir and buprenorphine/naloxone has been assessed in two PK studies, one in healthy volunteers (13 subjects), and one in patients on stable buprenorphine/naloxone OST (12 subjects). Elbasvir/grazoprevir did not significantly affect the PK of buprenorphine, norbuprenorphine or naloxone. Similarly, the OST had no impact on the PK profiles of EBR and GZR.[81]

The DDI between glecaprevir/pibrentasvir and methadone or buprenorphine/naloxone has been assessed in a PK study in 23 subjects on stable OST (11 on methadone, 12 on buprenorphine/naloxone). No significant impact of glecaprevir/pibrentasvir on methadone and buprenorphine/naloxone PK was observed. The exposures of glecaprevir and pibrentasvir when administered with methadone or with buprenorphine-naloxone were marginally lower than those observed in other studies of glecaprevir and pibrentasvir administered alone in healthy subjects, which could be explained by the reduced rate of gastric emptying induced by opioids, decreasing the absorption of glecaprevir and pibrentasvir. However, these changes in the PK of glecaprevir and pibrentasvir were not considered as clinically significant.[82]

The lack of clinically relevant DDI between glecaprevir/pibrentasvir and OST has been recently confirmed based on data from eight international phase 2 and 3 trials of glecaprevir/pibrentasvir. Among 2256 enrolled patients, 157 patients (7%) were on OST, with 76% receiving methadone. With similar adherence frequencies between OST and non-OST patients (98% and 99% respectively), the SVR 12 rates were high in both groups (96.2% in OST vs 97.9% in non-OST

patients). The safety profile was comparable, with a modestly higher percentage of patients on OST experiencing AE considered as possibly related to the study drugs (48% vs 40%).[83]

We did not find published PK studies on DDI between opioids and sofosbuvir-based combination. The lack of effect of sofosbuvir on methadone PK was already reported in the EMA reports of Sovaldi®.[84] The risk of a clinically relevant DDI with the addition of voxilaprevir is low.[34] In a pooled analysis of phase 3 studies, 194 patients (4%) were on OST (113 on methadone, 75 on buprenorphine): the SVR 12 rates were high in both groups (94% in OST vs 97% in non-OST patients). The rates of AE were similar in both groups (78% vs 77%). These results suggest that sofosbuvir-based therapies are effective and safe in patients receiving OST.[85] A Canadian cohort study that enrolled 5283 eligible PWSUD treated by sofosbuvir/ledipasvir (n finally treated = 3413) or sofosbuvir/velpatasvir (n treated = 1574) showed that patients with injection drug use (IDU) were less likely to achieve SVR as compared to other groups without IDU (adjusted odds ratio: 1.91 in patients not on OST, and 1.50 in patients on OST). The lower observed SVR among PWID was related to higher loss to follow-up, with a part of this loss related to deaths from drug overdose, rather than detrimental interactions between DAA and OST and/or drugs of abuse.[86] Another cohort study compared the efficacy of DAA (sofosbuvir-based therapy as well as paritaprevir-ritonavir/ombitasvir ± dasabuvir and elbasvir/grazoprevir) in patients with or without a history of injecting drug use: among the 1752 patients enrolled, 47% reported no history of injecting drug use and 53% were PWID, with 42% not on OST and 11% on OST (mainly methadone). This study confirmed that the SVR rates were lower among PWID (92% in PWID not on OST, 89% in PWID on OST) compared to patients without a history of injecting drug use (95%). This observation was mainly attributable to higher rates of discontinuations after the occurence of AEs and, especially, loss to follow-up in PWID, and not to virologic failure.[87] The likelihood of potential interactions issues is not plausible.

5.2 | Interactions of DAA with stimulants

No detrimental outcome associated with potential interactions of DAA with stimulants was reported. Based on their respective metabolism and clearance, a clinically significant interaction is unlikely (see the website https://www.hep-druginteractions.org/checker).

5.3 | Interactions of DAA with ethanol

High-risk alcohol consumption was recognized as a factor associated with lower adherence to antiviral treatment and increased propensity of failure with former interferon and ribavirin treatment.[88] Few comparable data are available with the DAA.[89] Some authors suggested that alcohol consumption may be a risk factor in a lower response to DAA, through different mechanisms: decreased susceptibility of viruses to DAA and decreased immune response to eliminate remaining viruses.[90]

A study compared the SVR of 15 151 veterans treated with DAA according to alcohol consumption. The DAAs were as follows: sofosbuvir, ledipasvir/sofosbuvir or ombitasvir-paritaprevir-ritonavir and dasabuvir. Most patients were abstinent (10 387, 69%), while 3422 (23%) had low alcohol consumption, and 1342 (9%) had high-risk drinking. The proportion of patients with SVR was comparable between the three groups: 91.9% (95% CI: 91.3-92.5) vs 93.2 (95% CI: 92.2-94.1) vs 91.4% (95% CI: 89.5-92.9). These results suggested that the response to DAA remained regardless the level of alcohol use.[91]

A German registry study assessed the impact of alcohol and cannabis consumption on the efficacy of DAA (sofosbuvir-based therapy and paritaprevir-ritonavir/ombitasvir ± dasabuvir). Among the 7747 enrolled patients, 1015 reported alcohol consumption, and 631 of them were not on OST and did not use injected drugs. In these non-OST non-IDU patients with high alcohol consumption, the SVR rates were lower (85%) than in patients consuming no or less than 30 g/day (women) or 40 g/day (men) (91%-92%). Regarding cannabis consumption, SVR rates did not differ between the different patient groups.[92]

6 | DISCUSSION

The interactions between DAA and substances of abuse have been assessed in four PK phase 1 studies, all involving opioids.[80-82] Even though a wide range of phase 3 studies including DAA were performed and taken into account in the review, we did not identify any adverse outcome associated with PK/PD interactions. PK studies with opioids were performed in a limited number of healthy volunteers or patients on OST, whereas no specific PK data on ethanol and stimulants were found. Hence, our hypothesis on the lack of interaction with alcohol and amphetamine derivatives is derived from in vitro PK considerations. Clinical data involving higher numbers of patients were mainly based on post hoc and pooled analysis of phase 2 or 3 studies, whose primary endpoint was SVR.

In PWID (ie 0.34% of the total population in Western Europe), the prevalence of HCV is significantly higher (up to 50%).[93] PWID are a key cluster and reservoir because of the interindividual dynamics of HCV transmission. However, only approximately 50% of PWID in Switzerland and in other European countries were adequately screened in the past with at least one antibody test, followed by the HCV RNA quantification if tested positive for antibodies.[94]

An increased access screening program using rapid antibody saliva test and dried blood spot testing to the PWID population is likely to be highly cost-effective, since the increased uptake of DAA could achieve significant reduction in this vulnerable population. (Incremental Cost-Effectiveness Ratio per Quality-Adjusted Life-Year (QALY): USD 8337 – net monetary benefit USD 99 192 per person).[95] At a willingness-to-pay threshold approaching USD 100 000 per QALY, an increased access screening program linked to treatment scale-up with DAA is expected to have a 97.0% probability of being cost-effective compared to standard screening methods.

Treatment scale-up with DAA was identified as a requirement to achieve a significant reduction in HCV prevalence in

European countries, including custodial setting as a virus reservoir.[96] Similarly to the PWID population, comprehensive screening strategies in detention centres are likely to be very worthwhile, with 82.3% probability of cost-effectiveness. Extended screening strategies in the largest custodial setting of Switzerland are expected to achieve positive HCV-RNA identification in 63% instead of 35% of detainees and 117 instead of 65 cures per year compared with current practice.[97]

Associations between blood-borne viral (BBV) diseases and severe mental illnesses were identified and more precisely documented. Before HIV (odds ratio – OR = 2.57) and HBV (OR = 2.29), HCV (OR = 6.18) appears to have the highest risk in people having severe mental illness in Sweden.[98] A systematic review and meta-analysis indicated that pooled prevalence of HCV in people with mental illness was as high as 17.4% in North America (HIV 6%; HBV 2.2%) and 5% in Europe (HIV 1.9%; HBV 2.7%).[99]

In psychiatric patients, the potential for interaction with DAA is particularly critical because of dose-dependent and life-treating AEs, such as *torsades de pointes* associated with long QT.[100] In psychiatric setting, drug-induced long QT patients had more prevalent HCV infection (41.9% vs 9.8%, $P < .001$) associated with additional T-wave abnormality frequencies (35.5% vs 15.4%, $P = .003$).[101] Independent predictors for long QT tend to cluster and correlate indirectly with chronic HCV infection, such as drug abuse (or uncontrolled intravenous administration), and opiate maintenance program.

This finding is likely explained by the higher number of prescribed drugs in patients with severe mental illness and HCV infection. Altered liver function is further an independent risk factor to develop repolarization abnormalities, including drug-induced long QT. Since the 1970s, patients under a methadone maintenance treatment appeared to have a lower heroin overdose mortality, lower probability of relapse, blood-borne infections (including chronic HCV infection) and criminal activities compared to those who did not receive treatment.[102] Methadone is largely prescribed as maintenance treatment in patients suffering from dependence since it is considered as the most effective opiate substitution.[103] In psychiatric patients and PWID receiving methadone in addition to psychotropic medications (antidepressants, antipsychotics), an impaired liver function (with consecutive reduced cytochromes P450 enzymatic activity) increases the likelihood of DDI and long QT interval to occur.

Moreover, methadone appears to be more effective than other opiate maintenance treatments in retaining patients in cares.

In a cohort study of 1648 patients over a 3-year period, HCV co-infection nearly doubled the propensity of QTc of 470 ms or greater in patients with HIV infection (29.6% vs 15.8%, $P < .001$).[104] In contrast to HIV infection, it is not known whether the HCV viral load is associated with QT interval lengthening. Further, there are associated immunological mechanisms, such as liver kidney microsomal type 1 antibodies triggered by the HCV with reduction in the CYP2D6 activity, the most significant metabolic pathway that metabolizes a wide range of antipsychotics and antidepressants.[105]

A still ongoing case-control study in the largest custodial setting in Switzerland shows a linear correlation between QT interval and methadone dose (personal communication). Initial analyses of the preliminary results suggest that methadone dose, HCV infection and patient age may have an influence that could provide further insight to envision indirect benefit of DAA, to optimize screening and medical management of vulnerable population. There is neither increased propensity of adverse drug reaction nor reported repolarization disorder (eg long QT) in detainees taking DAA.

Overall, it is reasonable to assume that treating chronic hepatitis infection with DAA does not significantly increase the risk of both PK and PD interactions. In high-risk patients such as PWIDs or patient with severe mental illness, the risk of serious drug AEs by adding DAA appears limited. It is expected that screening and treatment of chronic hepatitis C infection as early as possible before hepatic dysfunction are not only cost-effective (limited transmission and complication) but also safe even in the presence of QT interval lengthening drugs.

7 | CONCLUSION

The HCV prevalence is significantly elevated in detainees, patients with severe mental illness and PWID. Marginalized populations represent key clusters and reservoir with high dynamic interindividual BBV transmission. These populations further consume a wide range of medications and illicit drugs. Efficacy and safety are paramount during the prescription of DAA. Based on our literature review with PK and PD consideration, the interaction potential of DAA with most opioids and illicit drugs appears rather marginal. Taken together, most DAA mainly inhibit the transmembrane transporter P-gp and to a lesser extent the metabolizing enzyme subfamily CYP3A. Although, theoretically, interactions are plausible with opioids including methadone, these were not confirmed in PK studies. Large phase 3 and cohort studies did not show any clinically significant SVR decrease in PWID taking DAA.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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Part 5

Natural language processing in drug safety surveillance

Introduction to the article

“Foufi V, Ing Lorenzini K, Goldman JP, Gaudet-Blavignac C, Lovis C, Samer C. Automatic Classification of Discharge Letters to Detect Adverse Drug Reactions. Stud Health Technol Inform. 2020 Jun 16;270:48-52.”

This article describes the application of NLP techniques to detect the presence or absence of ADRs in discharge letters written in French. We selected 300 hospital discharge letters. One hundred were positive letters in which a serious ADR was documented. They were selected based on a specialized consultation from clinical pharmacologists and manually annoted by them for the following categories: drugs, ADRs, trigger words (e.g. stopped, suspect). Two hundred were negative letters (absence of ADR). A supervised learning approach was then applied for the automatic classification of the discharge letters into positive or negative. Two of the machine learning algorithms (Naïve Bayes and Linear Classifier) showed good performances in terms of precision and recall. Our study shows that automatic processing of discharge letters can help identify patients displaying ADR during their hospital stay.

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Automatic Classification of Discharge Letters to Detect Adverse Drug Reactions

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Abstract. Adverse drug reactions (ADRs) are frequent and associated to significant morbidity, mortality and costs. Therefore, their early detection in the hospital context is vital. Automatic tools could be developed taking into account structured and textual data. In this paper, we present the methodology followed for the manual annotation and automatic classification of discharge letters from a tertiary hospital. The results show that ADRs and causal drugs are explicitly mentioned in the discharge letters and that machine learning algorithms are efficient for the automatic detection of documents containing mentions of ADRs.

Keywords. Adverse drug reaction, pharmacovigilance, text mining, document classification, supervised machine learning

1. Introduction

Adverse drug reactions (ADRs) affect 7 to 17% of hospitalized patients [1,2] and can result in serious morbidity, mortality and high costs. They are largely underreported, making active pharmacovigilance useful. The detection of ADRs can be performed through the review of electronic medical records (EMR) [3] or regular ward visits by a trained health professional [4], which can be time-consuming. In this context, data mining techniques, focusing on the automated identification of ADRs from the patient EMR can be helpful [5,6]. These techniques include structured data analysis as well as text mining, including natural language processing (NLP) [7,8]. In this context, techniques for the automatic classification of clinical documents have been proven effective [9–11].

The aim of this study is to assess the feasibility of using NLP techniques to detect the presence or absence of ADRs in discharge letters written in French and extracted from patients hospitalized in a tertiary hospital via a hybrid –machine learning and rule-based– method. In this paper, we will present the supervised learning method. Particularly, three machine learning algorithms for document classification have been applied and evaluated. For the creation of the training and test datasets, manual processing of 300 discharge letters was performed. The results show that ADRs are reported in the documents and that NLP tools are efficient for their automatic detection.

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2. Method

2.1. Data collection

Our study was approved by the local ethics committee (study number: 2016-02107). Hospitalized adults for whom a specialized consultation from clinical pharmacologists (in 2015 and 2016) had identified the occurrence of serious ADRs, during or leading to hospitalisation, were included in the study. Out-patients and cases of non-serious ADRs were not included. Based on these criteria, a dataset of 100 positive discharge letters (presence of ADR) and 200 negative letters (absence of ADR) was constituted.

2.2. Data processing

2.2.1. Manual annotation

For the creation of the training and test datasets, an expert manually annotated 100 discharge letters (positive dataset) and validated 200 letters (negative dataset). Based on specific guidelines, sequences of the following categories were annotated:

1. Drugs

The drugs category is divided in 3 sub-categories: a) commercial names, b) international nonproprietary names (INN), c) therapeutic class.

2. ADRs

Occurrences of ADRs and their consequences, symptoms, laboratory values are annotated. ADRs are divided in 3 sub-categories: a) names (hépatite/*hepatitis*), b) periphrases (perturbation des tests hépatiques/*liver test abnormalities*), c) characteristics (hémoglobine à 75 g/l/*haemoglobin at 75 g/l*).

3. Trigger words

Words like imputabilité/*causality*, stoppé/*stopped*, suspect/*suspect* that imply the presence of an ADR are annotated.

4. Drug indications

Indications for drugs entailed in ADRs are annotated.

2.2.2. Automatic classification of discharge letters

For the automatic classification of the discharge letters into positive or negative, a supervised learning approach was followed. The dataset is composed of the positive letters containing at least one annotation of ADR and the negative letters validated by the expert. For this task, three machine learning algorithms widely used for text classification tasks were applied: Support Vector Machine (SVM), Naïve Bayes Classifier, and Linear Classifier. From the whole dataset, 80% was used for training and 20% for testing.

3. Results

3.1. Manual annotation

Out of 100 letters of the positive dataset, 87 letters contained at least one annotated sequence. The mean length of a discharge letter is 785 words. In total, 1471 sequences were annotated. These results are summarized in Table 1:

Table 1. Number of occurrences per annotation category.

Annotation category	Number of occurrences	Unique occurrences
Commercial name	170	76
International nonproprietary name	210	87
Therapeutic class	126	59
Trigger word	293	156
ADR	441	217
Characteristic	130	103
Drug indication	37	28
Periphrasis	64	35

From the 200 discharge letters considered as negative (absence of ADR), 47 reported an ADR and 2 were empty. Therefore, the final negative dataset consists of 151 letters.

3.2. Automatic document classification

Three well-known classification methods implemented in the Scikit-learn python library [12] were applied and compared: Support Vector Machine, Naive-Bayes, and Linear Classifier. For the SVM model, the radial basis function was selected with a 'scale' gamma. The Multinomial Naive-Bayes was trained with default parameters. Eventually, the Linear Classifier is based on a stochastic gradient descent with a lower stopping criterion than default (tol=1e-6). Figure 1 represents the classification accuracy of each model after 50 iterations. For each iteration, the test set represents 20% of the whole corpus, i.e. 17 positive documents and 30 negative documents (k-fold = 0.8). The training time is ~100ms for each SVM iteration whereas it takes 2mn for both the Naïve Bayes and the Linear Classifier.

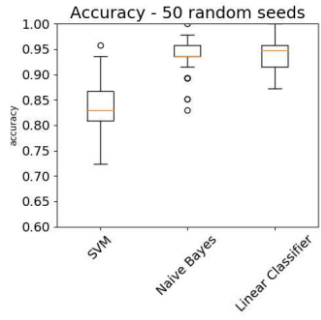

Figure 1. Mean accuracy over 50 iterations.

The SVM classifier achieved 0.83 accuracy, Naïve Bayes achieved 0.94 accuracy and the Linear Classifier 0.94. Complete results are shown in Table 2:

Table 2. Evaluation results of the automatic classification.

Classification method	Class	Precision	Recall	F1 score	Test set
SVM	Positive	0.80	0.97	0.88	17
	Negative	0.92	0.60	0.73	30
Naïves Bayes	Positive	0.93	0.97	0.95	17
	Negative	0.95	0.88	0.91	30
Linear Classifier	Positive	0.97	0.93	0.95	17
	Negative	0.90	0.95	0.92	30

The confusion matrices in Figures 2-4 display the performance of each classifier at the classification task:

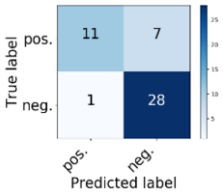

Figure 2. SVM.

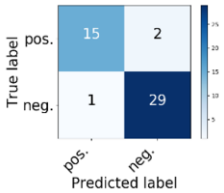

Figure 3. Naïve Bayes.

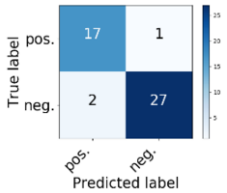

Figure 4. Linear Classifier.

4. Discussion

The manual annotation of positive discharge letters by an expert showed that ADRs were explicitly mentioned in most cases (>80%). A significant bias is that the test population were patients who had already received a specialized pharmacology consultation that had identified the ADR, thereafter mentioned in the discharge letter. Drugs were almost equally mentioned as commercial names, INN and therapeutic classes, and this has to be taken into account for their automated detection, especially given the diversity of commercial names in different countries. Trigger words were frequently present (293 occurrences); therefore, they constitute useful tools for the automatic detection of ADRs. ADRs were most frequently mentioned as plain terms, such as MedDRA (Medical Dictionary for Regulatory Activities) derived terms (i.e. *hepatitis*), but were also described as periphrases or as laboratory characteristics in many cases (approximately 200 occurrences) which makes their automatic detection challenging given the fact that the distinction between the 3 sub-categories was not always straightforward even for the human annotator.

For the automatic classification task into positive and negative discharge letters, three machine learning algorithms were applied and evaluated on the dataset. Naïve Bayes and Linear Classifier achieved the same mean accuracy over 50 iterations (0.94) and high precision and recall (Table 2).

A major limitation of this study is that the dataset was manually processed by only one annotator. Also, the classifiers should be applied and evaluated on a larger dataset.

5. Conclusion

In this study, we presented the methodology used for the manual and automatic processing of discharge letters generated in a tertiary hospital in the aim to automatically detect the presence or absence of ADRs. A dataset of 300 discharge letters written in French was manually processed and the output was used to train and test three machine learning algorithms for document classification. After comparison, we concluded that Naïve Bayes and Linear Classifier performed better than SVM at this task.

The manual annotation of the dataset from another expert will serve to create a gold standard corpus. In a next step, the trigger words and sequences describing the presence of ADRs identified during the manual annotation will be included in the rules that are being developed for the automatic identification and extraction of ADRs and their relations with causal drugs. Then, the hybrid method –machine learning and rule-based– will be applied and evaluated on the gold standard dataset.

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Part 6

Conclusion and perspectives

Conclusion

In conclusion, this thesis provided an overview of different approaches that are all useful to continuously monitor the safety profile of human medicines. Although many preclinical and clinical studies are performed during the drug development and allow to characterize the global pattern of adverse drug reactions (ADR) associated with a therapeutic compound, postmarketing surveillance is essential to detect rare ADR and those occurring after long-term use. The safety profile in neglected populations such as children is also often completed after the drug has been launched on the market. Other ADR influencing factors such as pharmacogenetic polymorphisms or drug-drug interaction are also often studied during the postmarketing phase. Among the existing tools used in postmarketing pharmacovigilance, this thesis illustrated the value of individual case safety reports. Although case reports and spontaneous reports have often been subject to criticism, they remain the cornerstones of pharmacovigilance and one should remind that most of the drug withdrawals occurring in the last decades were consecutive to case reports or case series. We illustrated the case of very rare, atypical low-energy femur fracture occurring after years of therapy with bisphosphonates, drugs used for the prevention of osteoporotic fractures. Published cases of this new ADR led to a change in the product labeling. Nowadays, atypical femur fractures are recognized as a rare ADR of bisphosphonates. Postmarketing studies are another approach to complete the drug safety profile. As an example, we presented the case of an observational study conducted in the pediatric population which aimed to evaluate the safety profile of oseltamivir used to treat H1N1 influenza. We observed that neuropsychiatric adverse events occurred frequently and a trend toward an association with *ABCB1* genetic polymorphism was suggested. Indeed, *ABCB1* encodes the P-glycoprotein, an efflux transporter that limits xenobiotics cerebral penetration. Therefore, variant carriers might be more prone to central nervous system ADRs. Drug-drug interactions (DDIs) may also impact drug safety. For example, a drug (perpetrator) inhibiting another drug (victim) metabolism could lead to increased concentrations and toxicity of the victim drug. Conversely, a perpetrator causing induction of the victim drug elimination can lead to therapeutic failure, which is also an unwanted effect, although not truly an adverse effect from the strict definition point of view. We illustrated the case of potential DDIs between direct-acting antiviral drugs used to eradicate hepatitis C virus, and opioids and substances of abuse, which may often be used concomitantly. According to our literature review, the potential of interaction appears limited. Finally, data mining approaches are promising tools to help to detect ADRs in large amounts of data, such as in large pharmacovigilance databases of spontaneous reports, electronic health records, and social media. We performed a study on discharge letters using natural language processing showing good performances in terms of precision and recall.

We believe that all the discussed approaches are necessary to thoroughly document and monitor drug safety throughout the whole lifecycle of a drug.

Perspectives

The discussed approaches offer numerous research perspectives.

We discussed the value of electronic health records for pharmacovigilance. EHR can be used in several manners for studying ADR. They can be manually reviewed by experts such as clinical pharmacologists or pharmacists to detect clinically drug-drug interaction, adverse effects, and other drug-related problems. EHR can also be processed by automated detection methods focusing on either structured data (e.g. laboratory, prescriptions) or narratives (e.g. clinical notes, discharge letters). One of our perspectives has been to conduct a retrospective study based on a manual review of EHR in the context of opioids use and abuse. Analgesics, including opioids, are among the most frequently used drugs. During the last decades, opioid consumption has much increased in several parts of the world but mostly in the US. At the same time, an increase in opioid-associated morbidity and mortality has been observed (62), which is referred to as the opioid epidemic. In Europe, including Switzerland, pharmacoepidemiological data are scarcer than in the US. Therefore we conducted a retrospective study to evaluate whether the opioid crisis is of concern in Geneva University Hospitals (63). To assess the frequency of emergency division visits due to adverse opioid-related events, we systematically reviewed the EHR of patients presenting to the emergency division during two months in 2018. In opioids users presenting to the emergency division because of an ADR, the main patterns of ADR observed were injury (fall), gastrointestinal disorders, and nervous system disorders. Overdose and abuse or misuse were rarely observed (manuscript in preparation). This is a good study example of how EHRs are a rich source of clinical information for drug safety monitoring. However, in the specific case of the "main" adult emergency division, automatic methods would have been helpful to analyze the large amount of data (approximately 13000 EHR to manually review). One of the challenges in the present case was that drugs taken at home are not entered in the EHR as structured data (i.e. International Nonproprietary Names, brand name) but as free text, making their identification not always straightforward. Therefore, several approaches and tools are complementary in drug safety monitoring and manual expert reviews will always be useful. Our perspective is to couple these approaches to improve drug monitoring at our institution.

We discussed the interest of pharmacogenetics in drug safety, as well as drug-drug-gene interaction. One of our perspectives has been to conduct a retrospective study of our patients in whom pharmacogenetics tests (genotyping and phenotyping) have been performed in the context of drug safety and/or inefficacy. Genotyping tests of cytochrome P450 (CYP) and transporters have been available in our institution for almost two decades. So are CYP450 phenotyping tests. In 2014, a phenotyping method using small doses of probe substrates has

been implemented in our division, called the Geneva cocktail (64). Since then, these tests have been performed in approximately 500 patients seen in our consultation for various causes, ADR, inefficacy, preemptively before prescription of certain drugs, etc. We are currently evaluating whether genetic polymorphism, DDI, or drug-drug-gene interaction had an impact on drug safety or efficacy in these patients (manuscript in preparation). The combined results of genotyping and phenotyping tests allow determining the respective role of genetic polymorphism versus DDI (or combined effect) on the observed clinical outcome. Our perspective is to help to predict the combined effect of certain genotypes and concomitant drugs on CYP450 net activity. This could allow better management of dosing recommendations in clinical situations where a drug-drug-gene interaction is expected. In cases where point-of-care phenotyping tests or clinical experience are not available to anticipate the net effect of drug-drug-gene interaction, physiological-based pharmacokinetic (PBPK) modeling can be a useful tool. PBPK modeling is an approach aiming to evaluate drug exposure in virtual populations by integrating key drug and system parameters in a model. It can be particularly informative and helpful in complex clinical situations where polymorphisms, DDIs, and comorbidities are simultaneously present (65, 66).

The present thesis discussed the value of spontaneous reporting in identifying novel or rare adverse effects, which is of particular importance in the context of the recently launched COVID-19 vaccines. The fast vaccine approval has attracted great interest from the general public and healthcare professionals on the importance of pharmacovigilance. In our institution, we currently collect vaccine-related adverse events occurring in the hospital staff. The vaccinated staff spontaneously report the adverse events they may experience to the occupational physicians of our institution. Occupational physicians then report these cases to our division for pharmacovigilance purposes. In this case, the hospital staff becomes patients that spontaneously report their experience with the vaccine. This illustrates the role of patients as partners in the context of pharmacovigilance. One of the potential perspectives would be to evaluate the frequency of vaccine recipient experiencing adverse events which are perceived as sufficiently serious to be spontaneously reported, from a hospital staff perspective. In this particular case, the denominator is known (number of vaccine recipients) in contrast to classic spontaneous report systems. In Switzerland, under the Therapeutic Products Act, consumers may also report undesirable effects of drug therapy. In the European Union, a reform of the pharmacovigilance system in 2012 introduced the legal right for individual citizens to report suspected ADRs directly to the authorities. When compared to healthcare professionals, patients report different ADRs and can identify novel ADRs (67). The patients' perspective also offers a better understanding of the patients' experience of the ADR (68).

We cannot complete the present thesis without discussing the importance of postmarketing pharmacovigilance in identifying novel safety signals of COVID-19 vaccines. The current

COVID-19 pandemic context has led to the rapid development and approval of COVID-19 vaccines. In December 2020, UK was the first country to approve the vaccine developed by Pfizer and BioNTech (69). The normal process for developing a vaccine is broadly similar to drug development and takes 10 to 15 years (70). The currently marketing vaccines have completed only a few months of the pivotal trial before being approved. Therefore, yet unobserved adverse effects need to be closely monitored (71). Compared to other medications used to treat sick people, the general public has a low tolerance to adverse events following vaccination because vaccines are given to healthy persons to prevent diseases (72). To decide whether a vaccine can cause a particular type of adverse event, multiple factors must be considered, including temporality, size of the effect, coherence across multiple lines of evidence, and biological plausibility. The comparison of adverse event rates between vaccine recipients and a comparison group (background rate) is a key element of initial assessments of a safety signal (73). The early detection of signals allows regulatory agencies, where necessary, to respond quickly to ensure that the vaccine benefit-risk profile remains favorable (70). Anaphylaxis and atypical thrombosis are examples of such signals.

Some weeks after the approval of Pfizer's vaccine, the first cases of anaphylaxis were reported. The incidence was estimated at 1 in 100000 which is 10 times higher than the incidence reported with other vaccines. Anaphylaxis has been hypothesized as being caused by polyethylene glycol present in the lipid component of the vaccine (74).

In March 2021, several cases of thrombosis, including fatal cases, with or without thrombocytopenia have been reported after vaccination with the COVID-19 vaccine from AstraZeneca. The potential adverse effect had not been observed during the phase III study (75). This conducted the EMA's safety committee, Pharmacovigilance Risk Assessment Committee, to review this signal. The committee concluded that the benefits of the vaccine continue to outweigh the risk of adverse effects (76). Most of the countries that had suspended vaccination with this product then resumed its use, with age limitations in some countries as cases were mainly observed in relatively young people. For example, the use of this vaccine is recommended in people over the age of 55 in France (77). A more recent review of the available data by the EMA's human medicines committee has led to the conclusion that these cases of unusual thrombosis occurred with an estimated frequency of 1 in 100000 vaccinated people, and that the vaccine's benefit-risk balance remains positive (78). Tobaiqy et al performed a retrospective review of the spontaneous reports submitted to the Eudravigilance database from 17 February to 12 March 2021. Among 54571 spontaneous reports, the authors identified 28 cases of thromboembolic reports that included cerebral venous sinus thrombosis, pelvic vein thrombosis, deep vein thrombosis, and pulmonary embolism. More than half of their cases were aged 85 years and over (79). See et al reviewed the US cases of cerebral venous sinus thrombosis (CVST) associated with the use of another adenovirus vector vaccine, the product from Janssen/Johnson & Johnson, reported to the Vaccine Adverse Event Reporting

System, which is also a database for spontaneous reports. They identified 12 cases of CVST with thrombocytopenia reported as of April 21, 2021, all occurring in relatively young people, aged from 18 to less than 60 years (80). Finally, Smadja et al reviewed the cases of arterial and venous thrombosis reported to Vigibase after the use of three vaccines, the ARNm based products from Pfizer and Moderna, and the AstraZeneca vaccine until March 16th, 2021. A total of 2161 thrombotic events (795 venous and 1374 arterial thrombotic events) were reported (1197 for Pfizer, 325 for Moderna, and 639 for AstraZeneca) (81). Greinacher et al provided mechanistic insights on the pathogenesis of this vaccine-associated unusual clotting disorder. After a thorough assessment of 11 patients who developed thrombosis or thrombocytopenia after receiving the AstraZeneca vaccine, the authors demonstrated that 9 of these 11 patients tested positive for the platelet factor 4 (PF4) dependent platelet activation assay. They suggest that these cases of atypical thrombocytopenia resemble severe heparin-induced thrombocytopenia, although their vaccinated patients did not receive any heparin to explain the subsequent occurrence of thrombosis and thrombocytopenia. The authors propose to name this novel entity vaccine-induced immune thrombotic thrombocytopenia (82). Similar laboratory features were reported in five patients by Schultz et al (83).

We can distinguish between passive and active surveillance methods. Passive surveillance is mainly based on spontaneous reports to detect signals and relies on the participation of healthcare professionals. Active surveillance needs a more systematic collecting of data for example from both vaccine recipients and vaccinating healthcare professionals. For COVID-19 vaccines active surveillance is probably better to identify potential safety or effectiveness concerns with real-world use (70). In France, the drug regulatory authority put in place a specific strengthened surveillance system for the COVID-19 related ADRs aiming at early detection of potential safety issues to take rapid relevant risk minimization measures if necessary. The system includes a daily assessment of spontaneous reports by regional pharmacovigilance centers and the establishment of weekly pharmacovigilance reports for each marketing vaccine published on the French drug authority website (84). Our perspective is to develop such regular drug safety communications to be published on our hospital website. We recently started with communication on COVID-19 vaccines' latest safety news. We aim to regularly update this document but also to extend this concept to global drug safety communications.

In conclusion, pharmacovigilance and spontaneous importance has gained much importance and interest from the general public and healthcare professionals in this new era of COVID-19 pandemic. All available tools are useful and complementary for drug safety monitoring and for the patient's sake.

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