Role of abdominal and interventional radiology in multidisciplinary management of alcohol-related liver disease

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Role of abdominal and interventional radiology in multidisciplinary management of alcohol-related liver disease

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Summary

Alcohol-related liver disease is a major health problem, cause of extensive morbidity and mortality. From illness diagnosis to interventional treatment and imaging follow-up, radiology participates actively in the management of this disease, usually as part of a multidisciplinary medical staff. This thesis encloses a compilation of relevant scientific studies formulated, designed and conducted by the author in the field of diagnostic and interventional radiology for alcohol-related liver disease. The compilation demonstrates high quality of technical development for diagnostic improvement as well as innovations for disease-related interventional procedures. The author's studies contributed to radiology by providing new imaging features that represent additional patients' prognostic factors. Furthermore, the author also provided novelty in embolization material and endovascular technique for interventional procedures of patients with alcohol-related liver diseases. The relevance of these studies was confirmed by publication in prominent scientific medical journals. Researches similar to those presented in this thesis are essential to the progress of this medical specialty. Moreover, they are proof of the indispensable role of radiology for the management of this common disease.

Abbreviations

AAH	Acute Alcoholic Hepatitis
ALD	Alcohol-related Liver Disease
DUS	Duplex-ultrasound
EASL	European Association for Study of the Liver
HCC	Hepatocellular Carcinoma
IR	Interventional Radiology
(MD)CT	(Multidetector) Computed Tomography
MELD	Model for End-Stage Liver Disease
MRI	Magnetic Resonance Imaging
RFA	Radiofrequency Ablation
ROI	Region of Interest
3D	Three-Dimensional

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Introduction

Abdominal radiology is of great importance in the management of alcohol-related liver disease (ALD). From disease detection to lifetime follow-up, from treatment planning to treatment itself, the radiologist takes nowadays an active part in management of ALD. Moreover, radiology plays important role in estimation of prognosis and patient survival in different phases of follow-up. Recently, in addition to the ever-increasing precision and efficiency of diagnostic radiology, minimally invasive interventions is gaining greater acceptance as treatments of choice of chronic liver disease complications such as hepatocellular carcinoma (HCC) or portal hypertension. Intervention radiology also encloses important tasks in treatments that involve preparation to surgery or management of diverse unexpected post-surgical complications.

The recent developments in patient care management have also enlightened the importance of a multidisciplinary approach of pathologies. That is a transversal view of medicine that places the patient in the middle of the strategy of care and allows each specialist to contribute with his knowledge to the group (fig. 1). This new approach supported the creation of centers of expertise with multidisciplinary staffs were patient´s conditions are considered and discussed from the point of view of each medical speciality. In fact, it is usually the radiology that establish the connection and cooperation of the different specialities because it accounts for considerable charge in disease diagnosis, management, control of treatment and follow-up.

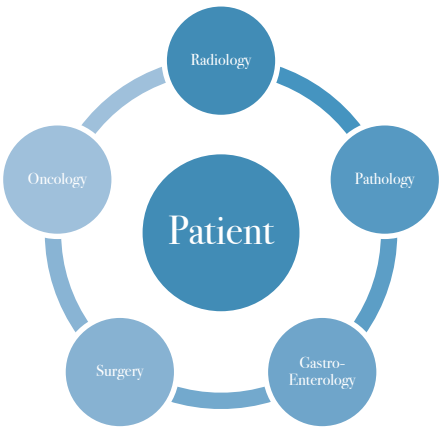

Figure 1. Medical specialities involved in multidisciplinary management of alcohol-related liver disease

The inextricable links between radiology and new technologies offers this specialty immense potential for development and innovation. Nevertheless, these innovations must pass through the filter of scientific research. Only the scientific studies will be capable to prove their safety, efficiency and usefulness for patients. The radiologist therefore has a fundamental role to play in the development of these new technologies as well as in the evaluation of their clinical usefulness.

This compilation of scientific studies provides exemplars of technical improvements in the field of diagnostic radiology and interventional radiology for the management of patients with alcohol-related liver disease. The appendix 1 demonstrates an article of diagnostic radiology computer development that establishes a correlation between numeric data of hepatic volume and a clinical score of prognoses. The appendix 2 exposes a study that determines an association between the presence of peribiliary cysts detected through liver imaging and degree of liver regeneration. Appendix 3 exposes a clinical study of interventional portal embolization before major hepatectomy in cirrhotic patients. It was the first study to illustrate the employment of ethylene vinyl alcohol copolymer to embolize hepatic

portal vein branches. Finally, in this collection, the Appendix 4 illustrates the role of interventional radiology to treat a specific complication after surgical treatment for cirrhotic patients. The study demonstrated the role of endovascular treatment of arterial stenosis after liver transplantation.

Herein all along this exposition, other articles also demonstrate the importance of the cross-cutting view of medicine and the close collaboration that exists between medical specialties. This is therefore a clear demonstration of the association of three objectives sought by the clinical centers of expertise, namely multidisciplinary team, advanced technology and medical research.

Alcohol-related Liver Disease

Alcohol-related liver disease is a major health problem. With the HEPAHEALTH project[1] of the European Association for Study of the Liver (EASL) we have a much better idea of the prevalence and epidemiology of alcohol-related liver disease across Europe. We know from this source that Europe as the largest burden of liver disease in the world and that it continues to grow[1][2]. Europe has the highest per capita alcohol consumption of any of the global WHO regions, and the men and women age-adjusted prevalence of cirrhosis has a median of 833 cases per 100 000[1]. Nevertheless the etiology of cirrhosis varies between regions, Switzerland is considered as a nation of Western Europe where alcohol is the first cause [1]. Adding alcohol with another risk factor such as obesity or hepatitis B or C strongly worsens the risk of developing a chronic liver disease[3].

Notably, alcohol-related liver disease is a leading cause of death among adults having excessive alcohol intake[2]. It is estimated that 2.3% of deaths occur due to alcohol-related liver disease worldwide[4,5]. The prognosis for patients with chronic alcohol induced liver disease is particularly poor – mortality rate is around 70% at 5 years and around 90% at 15 years[6]. In some European countries the mortality rate due to alcohol-related disease is as high as 47 each 100.000 habitants[7].

Switzerland has an elevated rate of alcohol consumption estimated in as high as twice the global average[8]. The tradition of alcohol consumption in Switzerland results in a total intake of 10.5 litres of pure alcohol per adult per year[9]. Consequently, alcohol has great impact in overall mortality in Switzerland. The single most important cause of death related to alcohol in Switzerland is alcoholic liver disease[8]. In younger ages (15-34 years-old), the most important cause of alcohol-related death is self-injury, but as age increases, liver disease becomes an essential cause.

The relation of alcohol abuse and liver disease is exponential, with excessive drinkers consisting of a large proportion of patients with alcohol-related cirrhosis[10]. The liver receives the furthermost impact of heavy drinking because it is the site of ethanol metabolism. The excessive and prolonged abuse of alcohol causes a large spectrum of liver diseases shifting from steatosis to hepatitis and finally to cirrhosis and its complications as hepatic insufficiency, cancer and acute gastrointestinal haemorrhage.

The mechanism of liver disease caused by alcohol is basically an oxidative stress-related hepatocellular necrosis and stellate cell-related fibrogenesis[11]. Ethanol is metabolized in liver by alcohol-desidrogenase enzyme, it catalyses

sequential oxidations that convert ethanol to acetate generating great amounts of various reactive oxygen species. Continuous production of these reactive molecules in heavy drinkers leads to the condition of oxidative stress. Free radicals and lipid peroxidation induce ballooning of hepatocytes and the formation of Mallory bodies. These Mallory bodies are responsive proteins originated from oxidative stress and they consist of anomalous keratins, ubiquitins and protein p-62. Ballooning hepatocytes induces tumour necrosis factor-alpha realise and formation of proinflammatory cytokines that cause neutrophilic infiltration and activation of Kupffer cells, the resident liver macrophages. In addition, activation of stellate cells increases matrix production and fibrogenesis[12]. The sustained fibrogenesis conducts to cirrhosis which is an irreversible remodelling of the hepatic architecture based on fibrosis and development of hepatic regenerative nodules. These nodules develop in response to continuous hepatic parenchyma injury and the cirrhotic liver is characterized by dysmorphy of the parenchyma and the vascular architecture. Finally, chronic liver disease may lead to several complications; the most feared being hepatocellular carcinoma (HCC) and portal hypertension.

Carcinogenesis in chronic liver disease is complex and generally a result of a long-term process related to a carcinogenic pathway into the regenerative nodules that progress to dysplastic nodules, subsequently to low-grade neoplastic nodules and finally to high-grade carcinoma. HCC is the most common primary malignancy of the liver. The connection between hepatocellular carcinoma and cirrhotic liver which function is compromised affects directly the therapeutic options because large hepatic resections might be contraindicated due to the restricted hepatic functional reserve. Consequently, any decision of treatment of HCC needs to be based also in life expectancy given by the cirrhotic status.

Portal hypertension is the result of increased hepatic flow resistance associated to increased portal inflow. Increase vascular resistance of the cirrhotic liver is due to mechanical parenchymal changes related to regenerative multinodularity and fibrosis leading to microvascular occlusion. Moreover, dynamic changes related to endothelial dysfunction results in elevated vascular tone. Increased portal inflow is associated to splanchnic vasodilatation which results from increased levels of nitric oxide usually observed in cirrhotic patients.

The Lancet Liver Commission acknowledged that delayed diagnosis of liver disease is probably the most important clinical concern related to cirrhosis[13]. Around 75% of patients with fatal cirrhosis were unaware of their disease until presenting signs of liver failure or variceal bleeding. Considering this, diagnostic radiology has an important role to play in detecting early signs of alcohol-related liver disease. Furthermore, diagnostic radiology and interventional radiology have crucial assignment in identification of potentially life-threatening complications of ALD as portal hypertension and hepatocellular carcinoma. As a result, advances in technical development of radiology might be of great impact in the management of alcohol-related disease.

The role of diagnostic radiology

The different imaging techniques used daily by the radiologist to explore the liver are computed tomography (CT), magnetic resonance imaging (MRI) and Duplex-ultrasound (DUS) (Fig. 2). The information drawn from these radiological techniques is mainly on the hepatic morphology. This use has now reached maturity, but it has been shown that it is possible to obtain a lot of other information

from these images either by exploiting digital information dynamically, as for example with the techniques of liver perfusion[14], or by directly exploiting raw digital information (texture analysis)[15]. This is where some of the latest research in abdominal imaging is directed, with the development of functional or structural imagery and, of now, radiomics[16].

Figure 2. Illustration of imaging techniques used routinely by the radiologist exploring alcohol-related liver pathologies. Upper left: CT control after a chemoembolization. Upper right: US control of the portal vein patency and direction of flow. Bottom left: fluoroscopic control of a tube placement. Bottom right: evaluation of the relation between an HCC and the biliary tree with MRI.

In the first research presented in this collection (appendix 1), the aim of the study was to test morphometric and radiodensity parameters extracted from CT

scans performed in patients suffering from acute alcoholic hepatitis (AAH) in order to assess their clinical prognostic value. As mentioned in the introduction, this article is an example of the potential of radiological examinations to provide further non-invasive and objective information, in addition to the radiologist's judgement, which depends on his or her level of training and experience. It could be a way to rapidly and objectively identify patients at high risk of death in order to provide them with adequate care without delay. Indeed, AAH is a clinical syndrome that occurs in a situation of exacerbated alcohol intake in a condition of chronic alcohol abuse. Steatohepatitis is the main cause of this inflammatory disease which destroys the hepatocytes and, in the absence of treatment, results in 35% to 50% of incidence of death within one month[17]. The advantages of computed tomography for these cases are the wide availability of tomography device and the good reproducibility of the calculation of liver volume[18]. Moreover, patients suffering from AAH often have a CT scan in order to exclude some differential diagnoses, even if in the current recommendations computed tomography is not part of the initial workup[19]. The idea of the research was therefore to verify the possibility of obtaining additional useful data from these imaging studies. The method used involved a retrospective analysis of 58 patients treated for histologically proven acute alcoholic hepatitis by transjugular liver biopsy. The patients had undergone pre- and post-treatment CT scans and those images were quantitatively analyzed by measuring the tissue attenuation coefficients of six regions of interest (ROI) of the hepatic parenchyma and three ROI of the splenic parenchyma. The aim was to obtain an attenuation index that defined the presence of hepatic steatosis[20][21]. We used the same radiological studies to calculate hepatic and splenic volumes. The last information that was taken from the scans was the body composition analyses. Using a semi-

automatic volumetric measurement program based on the different attenuation coefficient between tissues, we quantified the amount of intra-abdominal and subcutaneous fat, as well as muscle mass, normalized to the height and weight of the patients (Figure 3).

The results showed that the liver attenuation indices calculated, based on hepatic and splenic ROI, were significantly higher in patients who decreased their MELD score by at least 3 points at 3 months. The results also showed a relationship between the ratio of normalized liver weight to body weight and improvement in the MELD score. On the other hand, the subcutaneous fat index did not show significant differences.

This article consequently demonstrated that liver volume is a major positive prognostic factor in patients with acute decompensated alcoholic hepatitis, and that the malnutrition condition of the patients could be estimated. The CT scan is therefore a relatively simple method of analysis of body composition and great amount of information exploited on this type of examination may be implemented in clinical routine.

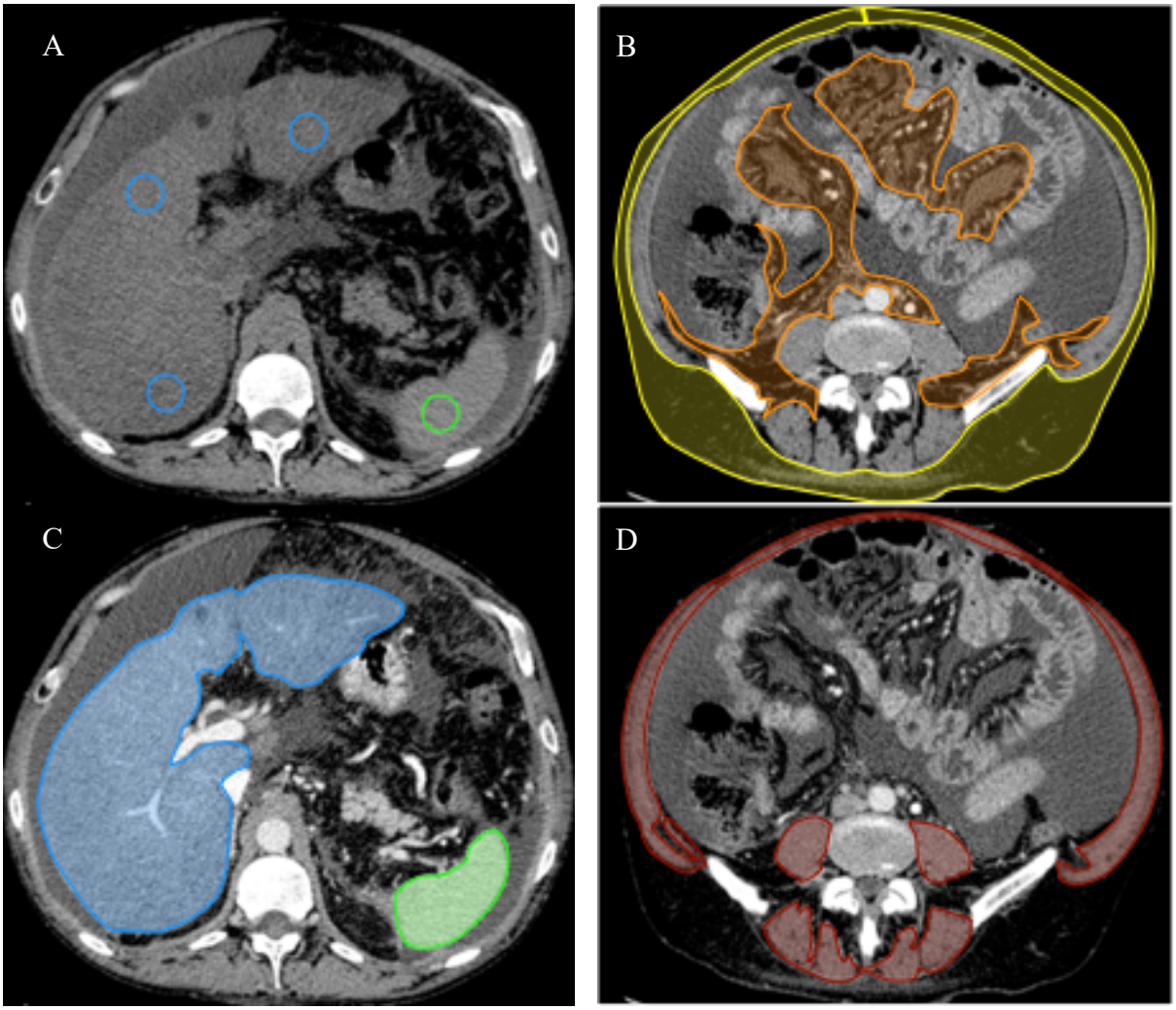

Figure 3. Computed Tomography images showing, ***A*** *regions of interest (ROI), three into the liver and one into the spleen. These ROIs are used to measure the attenuation coefficient which is correlated to the liver fat content.* ***B*** *Semi-automatic volumetry calculation of the subcutaneous and intra-abdominal fat.* ***C*** *An example of the volumetry of the liver and spleen and* ***D*** *volumetry of the muscle mass*

The second article presented here (Appendix 2) is an original research paper aimed to establish a link between the occurrence of dilated peribiliary glands in the liver, and a clinical situation; the presence of chronic liver disease. The method consisted of immunohistochemical and morphometric analysis of peribiliary glands from 71 liver explants from transplant patients combined with retrospective analysis of imaging and clinical data. Dilated peribiliary glands results in the formation of peribiliary cysts. It is assumed that the peribiliary cysts are the consequence of local inflammatory events that impair drainage of normal peribiliary glands to the bile ducts. In this research, the presence of histologically proven peribiliary cysts was associated with an increased MELD score, supporting the basic hypothesis that these cysts would result from attempts by the liver to regenerate in the event of liver failure. However, the study showed that imaging analysis had low sensitivity to detect peribiliary cysts and this factor reduces the impact of this imaging feature in current diagnostic radiology. Nevertheless, since the finding has high specificity, when it is present, it may aggregate valuable data to the calculation of patient prognosis.

Role of interventional radiology

Interventional radiology (IR) started to grow in the early 1970s, with the introduction of new puncture techniques, catheterization and embolization equipment [22]. Later came the metallic prostheses which made it possible to better treat obstructions, in particular biliary [23]. A little more recently, the second major innovation has been the development of percutaneous treatment of liver tumors, first with chemical agents like absolute alcohol or acetic acid[24], more recently with

physical agents like radiofrequency and microwaves[25], and in the near future with high frequency focused ultrasound (HIFU). Interventional radiology was particularly developed to respond to circumstances in which the surgical risk was too high to be taken, but where it was nevertheless necessary to treat the patient. Since then, IR has become increasingly ambitious and aggressive, while, at the same time, surgery has developed less invasive and sometimes ambulatory techniques to the point that today the difference between interventional radiology and minimally invasive surgery has become very tenuous.

Figure 4. Illustration showing different imaging techniques used routinely by the interventional radiologist treating alcohol-related liver pathologies. Upper left: microwave HCC ablation under CT control. Upper right: US guides a hepatic tumor ablation. Bottom left: selective chemoembolization under fluoroscopic. Bottom right: HCC ablation by radiofrequency under MRI guidance.

The role of interventional radiology in the management of alcoholic liver disease is wide. Firstly, it participates in the elaboration of the diagnosis via liver biopsy. Indeed, the histopathological analysis remains the gold standard for the diagnosis of liver cirrhosis [26]. Moreover, in oncological complications of ALD, such as HCC, histopathological analysis allows tumor characterization and grading, guiding the best treatment options for the patient [27]. IR allows these biopsies to be taken more safely while the risks of bleeding are greatly increased in these patients [28], either by using the transjugular route or by embolizing the biopsy track with glue. It takes also a role in the management of the bleeding complications themselves with transarterial embolization of the implicated vessel.

However, the major role of IR takes place in the treatment of two of the most serious complications of alcohol-related liver disease, portal hypertension and hepatocellular carcinoma. For example, interventional treatment options for portal hypertension includes transjugular intrahepatic portosystemic shunt (TIPS), balloon-occluded retrograde transvenous obliteration of varices (BRTO) and less frequently revision of surgical shunts [29]. With regard to the most feared complication, HCC, the role of the radiologist is multiple (Table 1) [30]. As mentioned before, it starts with the transparietal needle biopsy of the tumor under image control. Then in the treatment's options which can be curative or palliative. For the curative options, its role is either direct or indirect. Direct with thermo ablations techniques such as radiofrequency or microwave. Regarding EASL-EORTC clinical guidelines [31] these techniques are recommended in the treatments of very early stage or early stage HCC. These stages are defined, considering to the Barcelona Clinic Liver Cancer Stages (BCLC) [32] criteria's, as patients having a single tumor of less than two centimeters for very early stage, or patients having a single tumor of more than two

centimeters or less than three tumors less than 3 cm and which are not candidate for liver transplantation (Figure 5). IR can also play an indirect role in HCCs curative treatments by making an inoperable patient to become operable, like with portal embolization before major hepatic resection (Figure 6). Also, IR is capable to stabilize the disease while waiting for curative surgical resection or transplantation, like with pre-operative chemoembolization. But nevertheless, the major role of IR in HCC management is in palliative treatments, which still offer to the patients a possibility of treatment when a full recovery is not an option anymore (Figure 5). Nevertheless, relative and absolute contraindications for interventional radiologic treatments also have to be considered (Table 2).

Table 1. Procedures that the interventional radiologist can use to treat ALD's complications such as HCC or portal hypertension.

Procedure	**Purpose**	**Material**
Percutaneous Thermo ablation	Thermo-induced coagulative necrosis	Radiofrequency/Microwave/Cryo probes applied under imaging control (US/CT/MRI)
Percutaneous Chemoembolization	Cytotoxic-induced coagulative necrosis	Selective trans arterial injection of cytotoxic agents* potentiated by local ischemia
Selective Internal Radiotherapy (SIRT)	Radiation-induced necrosis and hypertrophy of the non-irradiated liver	Selective trans arterial injection of Y90** loaded microspheres
Portal Vein Embolization	Non tumoral liver hypertrophy allowing major hepatectomy Vascular stenosis/occlusion after surgery or hemostasis	Portal vessels embolization with hystoacryl (glue) or Ethylene vinyl alcohol copolymer (Onyx®) Desobstruction/stenting, hemostatic embolization (coils gelfo

*doxorubicine, indarubicine; **Ytrium 90

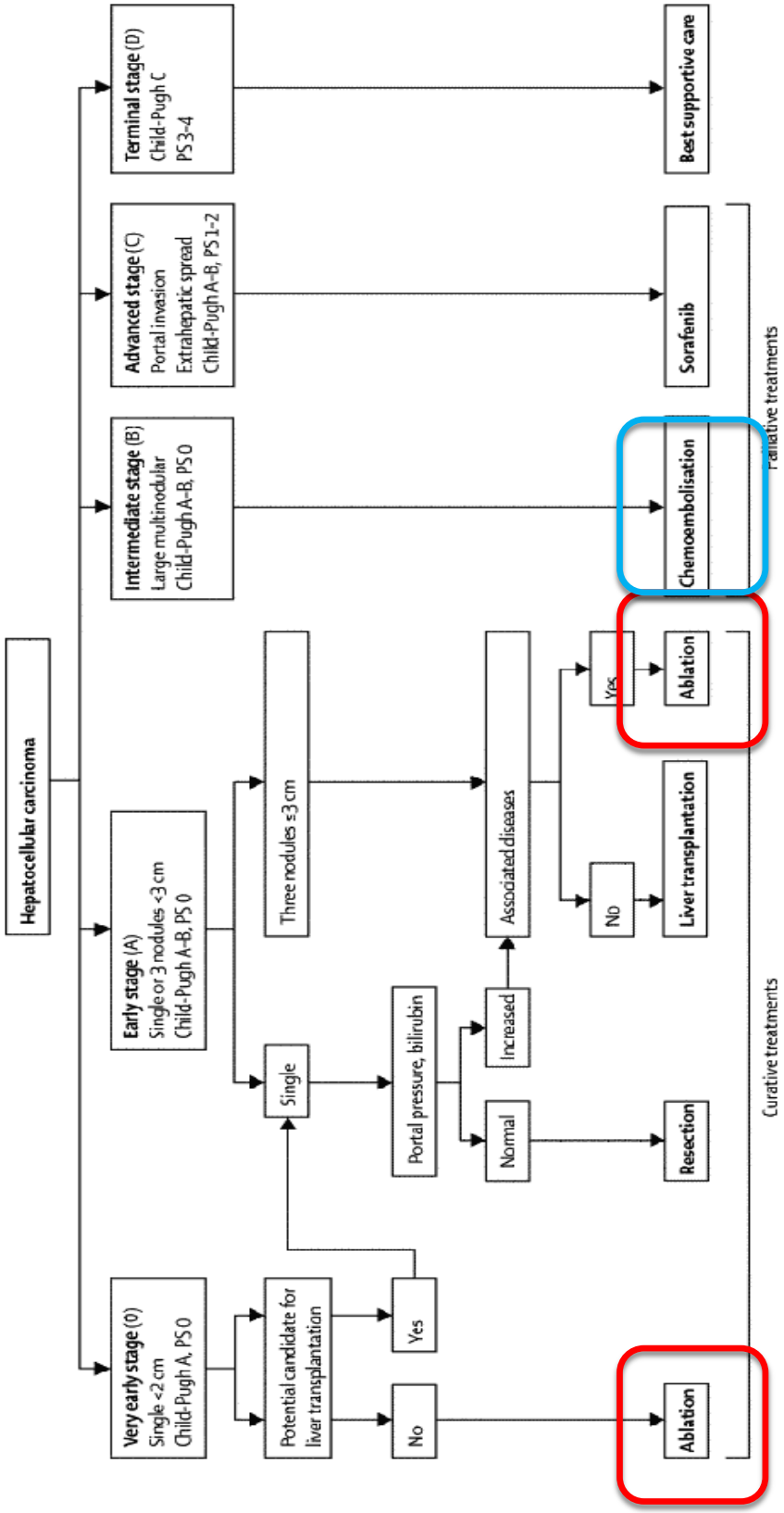

Fig. 5. EASL-EORTC clinical guidelines showing the place for thermo ablations techniques in the curative treatment of HCC (red squares), or palliative with chemoembolization (blue square).

Table 2: Relative and absolute contraindications for different interventional radiology therapy modalities for hepatocellular carcinoma

	Relative	Absolute
Percutaneous Ethanol Injection	• No intra-tumoural vascular shunts	• Large infiltrative tumours • Superficial tumours
Radiofrequence or Microwave Ablation	• More than 4 tumours • Tumour diameter 3–5 cm • Proximity to vessels and organs (organ displacement may be useful for protection)	• Multi-nodular tumours • Tumours > 5 cm • Tumour encasing hepatic bile ducts • Diffuse tumours
Transarterial chemoembolisation	• Tumour size ≥10 cm (sequential TACE of diverse tumour parts recommended)	• Extensive tumour growth invading both lobes • Severely reduced portal vein flow • Intolerance to chemotherapy • Occlusion of main portal vein
Radioembolisation	• Thrombosis or tumour obstruction of peripheral portal vein branches (main branch)	• Untreatable arteriovenous shunts • Extensive tumour growth (>40% of liver volume) • Severely reduced portal vein flow • Extrahepatic deposition of 99mTc-MAA on SPECT/CT • Lung shunt >20 % • Reflux into arteries that supply the gastroduodenal region

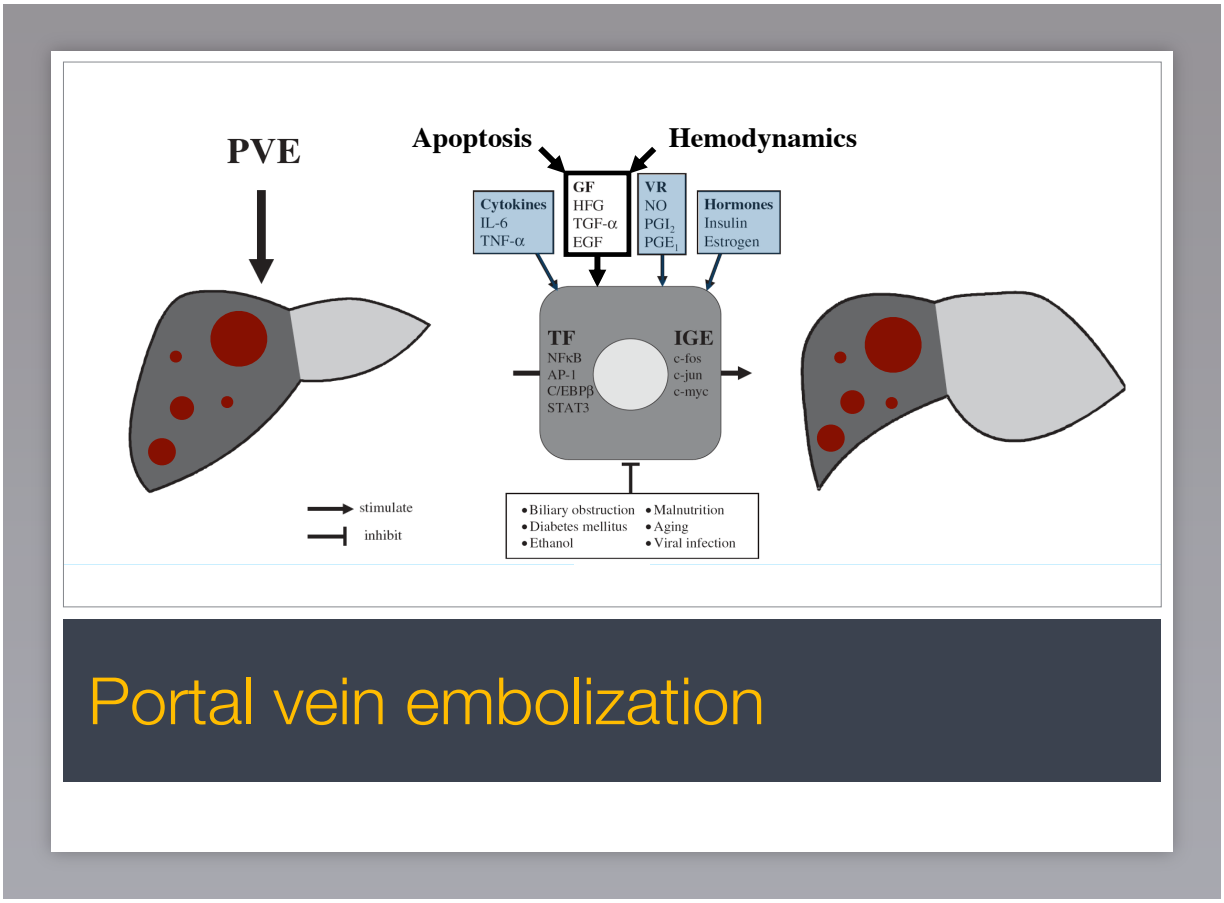

Fig. 6. Illustration depicting the principles on which are based portal vein embolization making non eligible patient becoming eligible for surgery after their future remnant liver grows sufficiently to allow normal liver functioning.

The next two articles presented in this collection are examples of the close cooperation existing between IR and surgery for the management of HCC.

Appendix 3 demonstrate a study that evaluated the efficacy and safety of selective injection of ethylene vinyl alcohol copolymer (Onyx ® Medtronic International Trading Sàrl Route du Molliau 31, 1131 Tolochenaz), a viscous radiopaque glue, in small portal branches at major risk of non-target embolization. Preoperative portal vein embolization is already a well-established interventional method to stimulate the hypertrophy of future liver remnant before partial

hepatectomy. This is necessary to avoid hepatic insufficiency after surgery. Although portal vein embolization is difficult and technically demanding. It is worth to mention that the risk of non-target embolization is considerable and if it occurs, posterior curative hepatectomy may be precluded. Therefore, the search of new materials and technical improvement of portal vein embolization is justifiable. Our research was the first study to evaluate the employment of ethylene vinyl alcohol copolymer for portal vein branches occlusion. Indeed, this research demonstrated that this embolizating material was easier to handle than the glue normally used in portal embolization n-butyl-2-cyanoacrylate (Histoacryl ® B. Braun Medica SA Seesatz 17, 6204 Sempach). In the condition of superselective embolization of hepatic segments, it would bring a technical benefit allowing embolization of portal branches normally not feasible, because of their anatomical origin starting from the branches which must be preserved at all costs to keep hepatectomy feasible.

The method consisted in the evaluation of 29 patients requiring extended hepatectomy to certain segments who were deemed at risk of unintended embolization and who therefore benefited from this particular procedure. The results showed that this technique of embolization was associated with additional enlargement of the residual liver and did not demonstrate unintended embolization. The conclusion was therefore that this method, inspired by neuro-interventional radiology techniques used in the treatment of cerebral aneurysms and arteriovenous malformations [33,34], was safe and feasible and useful to allow non-operable patients to become so.

The last appendix shows a study of both interventional and diagnostic radiology. The interventional part once again highlights the multidisciplinary side of the management of liver transplant patients. Even if in this study the indications for

liver transplantation were not only alcohol-related diseases, it was the most prevalent indication as expected in European countries [1]. The aims of this study was twofold, on one hand to judge the long-term permeability of endovascular treatment of post liver transplantation anastomotic arterial stenosis, and on the other hand to evaluate the fate of the bile ducts in these patients at high risk of bile duct necrosis, a dreadful complication requiring in the majority of cases a re-transplantation[35]. This study was both retrospective and prospective in the sense that the patients included had already been treated (minimum of one year, median of 5) in order to guarantee a long-term evaluation, and prospective in the sense that patients were recalled in the course of the study to judge the permeability of the hepatic artery and the state of the bile ducts. The results confirmed other data from the literature on the high immediate technical success of the endovascular procedures (94%), but also added new results on its long-term success. Indeed 80% of the arteries treated by endovascular techniques were permeable at 5 years. Another interesting result was that patients with early arterial complication (< 3 months after transplantation) probably developed more cholangiopathies than others, supporting the theory that a network of collateral vascularization can develop on liver grafts and therefor present a certain degree of protection for the bile duct [36,37]. Another observation was that having a normal duplex ultrasound exam was unrelated to having damaged bile ducts, supporting the idea that there is indeed a "sensitive period" during which bile ducts are likely to suffer more. This suggests implementing a policy of "screening" for vascular complications during the first tree month after transplantation.

Conclusion

The articles of this compilation represent major scientific investigation leading to more technical development and expansion of radiological applications in alcohol-related liver diseases.

With improvements in the capability of the different methods to image normal and pathological anatomy, superior medical diagnosis and better quality of disease staging are obtained. Moreover, advances in the field of interventional radiology lead to improved ability to care disease in different stages and routinely increase the radiological commitment into patient management.

The study on imaging evaluation of liver volume demonstrated the role of cross-sectional imaging as a prognostic tool for patients with alcohol-related liver disease. As widely known, this pathology presents as a broad spectrum of disorders, ranging from simple steatosis to end-stage cirrhosis. The gross morphology of the liver evolves according to the stage of liver disease. Gradual hypotrophy of segment IV and later of the right liver, associated to left liver compensatory hypertrophy are already recognized typical dysmorphic changes of alcohol-related liver disease. Nevertheless, abdominal CT imaging is constantly improving and our research added parameters to assess hepatic morphological changes and patients' nutritional status. Therefore, it promoted radiological compromise with patients' clinical evaluation by including new factors to estimate medical status and treatment outcome.

Advanced computational algorithms as presented here to calculate liver volume is characterized by a semiautomatic method. It is part of an artificial intelligence set that is in continuous progress nowadays and the tendency is to

become a completely automatic process. The recently introduced new terms radiomics and radiogenomics express radiological tasks that are in considering ever-developing advance. Radiomics represent the extraction of a high number of quantitative data from images and thereafter the combination of these data with clinical information. It is gaining each day more attention because this new knowledge may be used to guide medical decision and provide additional prognostic information. These data are undetectable at visual morphologic radiological analysis. Otherwise, radiogenomics identifies correlation between imaging features and tumor molecular data. These new radiological assignments are the evidence of imminent marriage between humans and machines into the future medical practice.

Morphometric calculations and attenuation coefficient parameters measurements extracted from scans, or information extracted from post processing programs using data-characterization algorithms as seen with radiomics, might in the future replace invasive measures such as biopsies. This could be of great benefice for patients suffering from alcohol-related liver disease, who usually present other pathological conditions as coagulopathy. Diagnostic imaging therefore has a central role to play in the development and research of alcohol-related liver diseases, notably by developing increasingly objective and quantifiable markers extracted from raw computer data and by using semi-automatic or artificial intelligence measurement tools [38]. As these tools turn into completely automatic procedures, time will be saved, and intra- and inter-observer variations will be eliminated. All of these progresses are part of a continuum of the medical management of diseases (Fig.7).

The second scientific study of this collection is another excellent example of the relationship between radiological feature and predictive patient outcome. The presence of peribiliary cysts on cirrhotic livers was demonstrated to be associated to the prevalence of liver failure. This was the first study to characterize pathologically the presence of dilated peribiliary glands in cirrhotic patients undergoing liver transplantation and correlate these findings with clinical markers of liver failure. Moreover, the research demonstrated an increased number of proliferative cells with stem cellular marker positivity in the lining epithelium of the peribiliary glands supporting that they contribute for the organ regeneration activity. Although imaging has low sensitivity to detect microscopic peribiliary cysts, large cysts may be identified specially in perihilar hepatic region as round or tubular periportal cystic structures. Quantification and measure of peribiliary cysts will be possibly subject to radiologist evaluation and therefore, a matter of intra- and inter-observer variation. However, in the future this might also be an imaging feature submitted to automatic segmentation and then, might be easily a part of clinic-radiological prognostic evaluation of patients with cirrhosis.

Portal vein interventions, particularly portal vein presurgical embolization, have great impact on the patients with alcohol liver disease complicated with HCC. New techniques involve meticulous understanding of the disease pathophysiology as well as knowledge about the characteristics of materials and computer devices. The new approach presented at this collection is of considerable therapeutic progress, although extended utilization in quotidian medical practice is still essential. Equivalent further research based on new material for portal vein embolization may proportionate additional advances in the field.

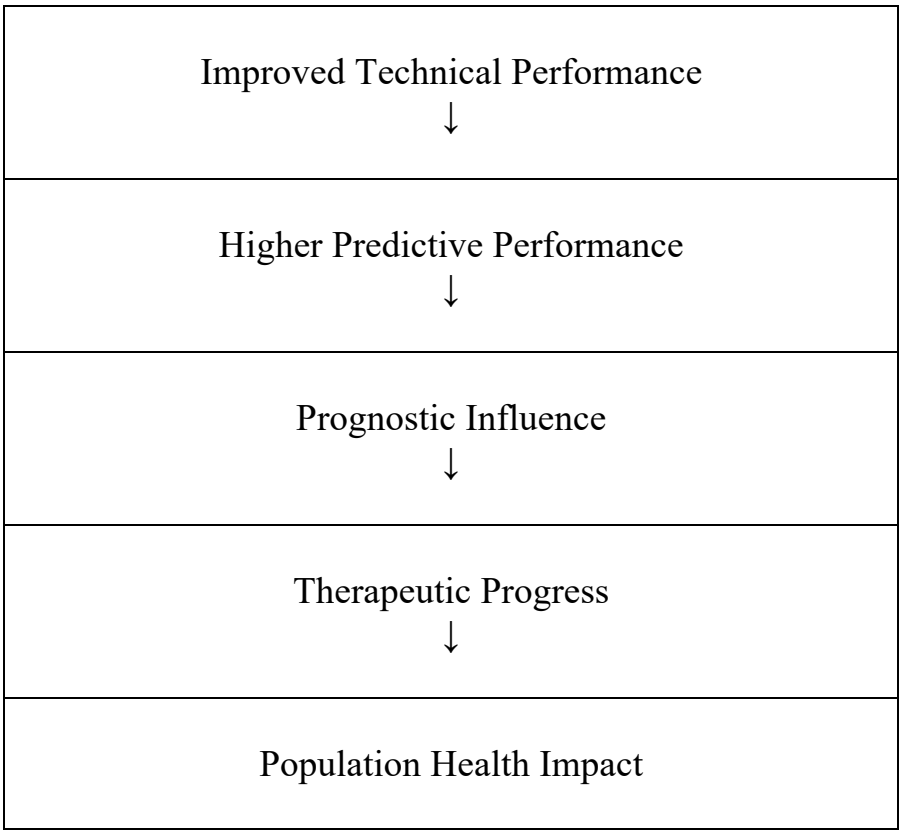

Figure 7. Flow chart outlining radiological advances effects on patients' prognostic and treatment approach.

The scientific study presented here on interventional radiology approach of patients after liver transplantation demonstrated that endovascular treatment for hepatic artery complications after surgery is feasible and safe. This is capable to improve patients post-surgical outcome since it reduces the risk of ischemic cholangiopathy. Prompt endovascular treatment of arterial complications after liver transplantation for cirrhosis may prevent the need of another large and complex surgical intervention and thus acting as supplemental factor for patient benefit.

For the physical part of the radiologist everyday practice, interventional radiology robotic tools are not yet available, but with liver biopsies in mind,

indications for invasive biopsy may drop in the future. Nevertheless, portal hypertension and the management of HCC will require the physical presence of an operator for a long time. Considering that we still see the early days of IR, the procedures might be less and less invasive in the future and this is a clear benefit for patients.

In conclusion, this thesis illustrates a great example of medical excellence and technological improvement and the importance of having a transversal vision of medicine encouraging close collaborations between specialties around the patient and his disease and the challenge it represents.

Appendix 1

Liver volume is a prognostic indicator for clinical outcome of patients with alcoholic hepatitis.

Summary:

The purpose was to evaluate the prognostic value of abdominal computed tomography in patients with alcoholic hepatitis. The methods of this ancillary study were based on data collected during a previous randomized controlled trial in patients with alcoholic hepatitis. Clinical response was defined as the improvement of the baseline MELD score ≥3 points at 3 months. All patients underwent contrast-enhanced CT of the abdomen. The following parameters were measured: (1) liver density, spleen density, and liver-to-spleen density ratio; (2) liver-to-body weight (LBW) ratio; and (3) subcutaneous fat, visceral fat, and muscular content. Improvers and non-improvers were compared with univariate, multivariate, and ROC analyses. Results were compared with a validation cohort of patients. The results were: fifty-eight patients (mean age, 56 years) were analyzed, including 34 (59 %) improvers. On multivariate analysis, LBW ratio (OR = 3.73; 95 % CI, 1.65-8.46; p = 0.002) and subcutaneous fat (OR = 1.01; 95 % CI, 1.00-1.02; p = 0.022) were associated with clinical response, with AUROC curves of 0.78 ± 0.06 (p < 0.001) and 0.66 ± 0.07 (p = 0.043), respectively. LBW ≥2.4 % predicted response with 88 % sensitivity and 63 % specificity. In the validation cohort (n = 42, 64 % improvers), the same cut-off value predicted response with 93 % sensitivity and 60 % specificity. In conclusion**,** in patients suffering from AH, the liver volume appears to be a major positive prognostic factor.

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Liver volume is a prognostic indicator for clinical outcome of patients with alcoholic hepatitis

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Abstract

Purpose: To evaluate the prognostic value of abdominal computed tomography (CT) in patients with alcoholic hepatitis (AH).
Methods: This ancillary study was based on data collected during a previous randomized controlled trial in patients with AH. Clinical response was defined as the improvement of the baseline MELD score ≥3 points at 3 months. All patients underwent contrast-enhanced CT of the abdomen. The following parameters were measured: (1) liver density, spleen density, and liver-to-spleen density ratio; (2) liver-to-body weight (LBW) ratio; and (3) subcutaneous fat, visceral fat, and muscular content. Improvers and non-improvers were compared with univariate, multivariate, and ROC analyses. Results were compared with a validation cohort of patients.
Results: Fifty-eight patients (mean age, 56 years) were analyzed, including 34 (59 %) improvers. On multivariate analysis, LBW ratio (OR = 3.73; 95 % CI, 1.65–8.46; $p = 0.002$) and subcutaneous fat (OR = 1.01; 95 % CI, 1.00–1.02; $p = 0.022$) were associated with clinical response, with AUROC curves of 0.78 ± 0.06 ($p < 0.001$) and 0.66 ± 0.07 ($p = 0.043$), respectively. LBW ≥2.4 % predicted response with 88 % sensitivity and 63 % specificity. In the validation cohort ($n = 42$, 64 % improvers), the same cut-off value predicted response with 93 % sensitivity and 60 % specificity.
Conclusions: In patients suffering from AH, the liver volume appears to be a major positive prognostic factor.

Key words: Alcoholic hepatitis—Computed tomography—Volumetry—Body composition—Prognostic factors

Abbreviation

AH	Alcoholic hepatitis
ASH	Alcoholic steatohepatitis
AUROC	Area under the receiver-operator characteristic curve
CI	Confidence intervals
CT	Computed tomography
LBW	Liver-to-body weight
MRI	Magnetic resonance imaging
OR	Odds ratio
ROI	Region of interest

Alcoholic hepatitis (AH) is a clinical syndrome which occurs in the setting of excessive consumption of alcohol and accounts for more than 0.7 % of hospital admissions in Western countries [1, 2]. Alcoholic steatohepatitis (ASH) is the predominant cause of this syndrome and is histologically characterized by liver inflammation in association with hepatocellular damage and steatosis, which often occurs against a background of established cirrhosis [3]. In severe clinical presentation, AH may

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progress to multisystem organ failure and infection, with a 28-day mortality between 35 % and 50 % in the absence of treatment [4]. Cessation of alcohol consumption is the cornerstone for the management of AH. First-line therapy in patients with severe biopsy-proven ASH includes corticosteroids [1]. Several scoring systems have been developed to assess disease severity and to estimate the short-term survival and to stratify patients in need of corticosteroids [5].

In chronic liver disease, the current role of cross-sectional imaging, including contrast-enhanced computed tomography (CT) and magnetic resonance imaging, is the morphological assessment of associated complications, mainly portal hypertension, portal vein thrombosis, or hepatocellular carcinoma [6]. CT offers several advantages, including rapidity of image acquisition, reproducibility, and better availability. CT is also able to provide a quick and an accurate estimation of the volume of abdominal organs, which may be of clinical value [7]. For instance, the spleen-to-liver volume ratio has been shown to be a very good predictor of significant portal hypertension [8]. Finally, CT may also offer an objective radiological assessment of body composition in patients with end-stage liver disease [9]. Recent advances in technology and reconstruction software have also allowed CT to non-invasively quantify focal or diffuse pathological processes in the liver and in the abdomen. Steatosis can be accurately estimated by measurement of hepatic CT attenuation [10]. Dual-energy CT with dedicated algorithms is able to precisely assess liver iron overload, or to discriminate patients with different extent of liver fibrosis [11, 12]. Finally, perfusion changes occurring early during fibrogenesis in chronic liver diseases can be detected with perfusion CT [13].

Currently, patients with AH undergo almost routinely liver and abdominal ultrasonography, but cross-sectional imaging is not recommended for the diagnosis or follow-up. Nevertheless, in the context of emergency, patients also frequently undergo abdominal CT to exclude differential diagnosis or detect associated complications. However, published data addressing its role are scarce, and the value of CT seems to be under considered at the early stage of decompensation. Thus, its predictive value in patients with AH remains unknown.

Therefore, the aim of this retrospective study was to determine whether abdominal contrast-enhanced CT provides imaging biomarkers independent of established clinical prognostic factors in patients with AH.

Materials and methods

Study cohort

The study was a retrospective analysis of clinical, biological, and radiological data, which had been prospectively collected during a recently published randomized controlled trial on treatment in decompensated AH [14]. This trial was approved by the institutional ethics committee (approval *n*° 07–145). Shortly after hospital admission and written informed consent, 58 patients with biopsy-proven ASH and cirrhosis were randomized to standard medical therapy (including alcohol abstinence, vitamin supplements, and oral corticosteroids when indicated) alone or combined with autologous bone marrow stem cells transplantation. The primary endpoint was a decrease of at least 3 points in the MELD score at 3 months. No statistically significant difference of clinically relevant improvement in liver function was observed between both groups. Based on this criterion, we subsequently classified all patients with a decrease of 3 points and more during these three months of follow-up as "improvers". The other patients with a decrease of less than 3 points of the MELD score were defined as "non-improvers."

Medical records

Age, sex, height, and weight were extracted from medical charts. The body mass index, the body surface area, and the MELD and ABIC scores were calculated [15, 16]. Transjugular liver biopsy with measurement of the hepatic venous pressure gradient was systematically performed at hospital admission [17]. Histological analysis of the liver biopsy specimen included a quantification of steatosis and a semi-quantitative scoring system for grading liver injury [18].

Computed tomography

At hospital admission and before starting treatment, all patients were examined with a four-phase contrast-enhanced CT of the abdomen. The same multidetector CT scanner (Somatom Sensation 64, Siemens Medical Solutions, Erlangen, Germany) was used with the following parameters: tube voltage, 120 kV; modulated tube current; gantry rotation time, 0.5 s; helical pitch, 1.20; and detector configuration, 64 × 0.6 mm. CT dose index volume and dose length product were recorded for each examination.

First, unenhanced imaging of the liver was acquired in an inspiratory breath hold. After intravenous injection of 2 mL/kg body weight of non-ionic contrast media (iohexol [350 mg I/mL]; Accupaque 350, GE Healthcare, Opfikon, Switzerland) at a flow rate of 4 mL/s using a power injector, followed by 30 mL of saline solution at the same flow rate, bolus tracking was started in the abdominal aorta with a threshold of 100 HU. Hepatic arterial, portal venous, and equilibrium phases were acquired 10, 45, and 150 s after bolus tracking, respectively. During hepatic arterial and equilibrium phases, imaging was acquired from the hepatic dome to the iliac

crest, whereas the entire abdomen was covered during the portal venous phase.

CT images were reconstructed using a standard filtered back-projection algorithm with the following parameters: slice thickness, 1.5 mm; slice interval 1.0 mm; matrix size, 512 × 512; and medium smooth-tissue convolution kernel (B20f).

Image analysis

CT images were analyzed both qualitatively and quantitatively with an open-source software (OsiriX 64-bit version 5.6; The Osirix Foundation, Geneva, Switzerland) by two radiologists (ST, RB) with 14 and 6 years of experience in abdominal radiology, respectively. The amount of ascites and portosystemic shunts was qualitatively classified in one of these categories, according to modified CT criteria for grading portal hypertension: none, moderate (fluid thickness, varices diameter less than 1 cm), or large (fluid thickness, varices diameter greater than 1 cm) [19].

On unenhanced CT images, six circular regions of interest (ROI) with a 1.5 cm diameter were placed within the hepatic parenchyma, avoiding vessels and artifacts: two ROI in the right lobe, two ROI in the left lobe, and two ROI in segment I and IV, respectively. Three ROI were placed at the superior, middle, and inferior parts of the splenic parenchyma with the same method. The different attenuation values were averaged to obtain the mean attenuation of the liver and the spleen. The liver-to-spleen attenuation index was calculated, with a cut-off value of 0.8 for the diagnosis of steatosis of 30 % or greater [10, 20].

The volumetric analysis was performed from the portal venous phase acquisition with manual contouring of the liver and the spleen, excluding the great vessels, the major fissures, and the gallbladder. The organ volume was calculated automatically, according to an algorithm that had already been validated [21]. The volumes of the liver and the spleen were normalized to the body weight, and the spleen-to-liver volume ratio was computed.

The analysis of body composition was performed at the level of L3–L4 disk space. The surfaces of the subcutaneous and the visceral fat were segmented using a brush ROI placed in the adipose tissue and was expressed in square centimeters [22]. The total adipose tissue and the subcutaneous-to-visceral fat ratio were computed. The surface of skeletal muscles was quantified using the same segmentation technique [23]. Since adipose and muscle masses are highly correlated with height of the patient, the total surface of fat and muscles were normalized for this parameter, respectively. Sarcopenia was defined on the basis of established cut-offs, which are 38.5 cm^2/m^2 for women and 52.4 cm^2/m^2 for men [24].

Statistical analysis

Statistical analysis was done using SPSS 15.0 statistical package (SPSS Inc, Chicago, IL). Continuous data were presented as mean (standard deviation) and range. Student's t test was used for comparisons between groups. Categorical data were expressed as frequency (percentage). Comparisons between groups were assessed using Fisher's exact or Pearson's χ^2 test. The statistical relationship between paired size measurements was studied with the Pearson's r correlation. The inter-observer agreement of continuous variables was assessed by calculating the intra-class correlation coefficients.

After univariate analysis, only the statistical significant covariates were kept for the binary logistic regression analysis (Wald test), and the adjusted odds ratio (OR) and their 95 % confidence intervals (95 % CI) were obtained. Receiver-operator characteristic curves were used to select cut-off values for continuous variables, and values with the best combination of sensitivity and specificity were chosen. The area under the receiver-operator characteristic curve (AUROC) was calculated for each variable. A p value of less than 0.05 was considered as statistically significant.

The clinical scores, anthropometric characteristics, and CT-based morphometric parameters were subsequently analyzed in an independent prospective validation cohort of 42 patients with biopsy-proven ASH and admitted consecutively to the same hospital institution. All patients from the confirmatory group met the same inclusion criteria aforementioned for the study cohort and were examined with contrast-enhanced CT of the abdomen before starting the treatment.

Results

Study cohort

The group of improvers included 34 patients (16 men; mean age, 55 years), while the group of non-improvers included 24 patients (18 men; mean age, 58 years). The mean CT dose index volume for all CT examinations was 77 ± 22 mGy (range, 47–129), whereas the mean dose-length product was 1812 ± 791 mGy·cm (range, 763–3651). The inter-observer agreement for CT measurements (attenuation values of hepatic and splenic parenchyma; liver and spleen volumes; surface of subcutaneous and visceral fat, and skeletal muscles) was excellent, with intra-class correlation coefficients ranging from 0.858 to 0.984.

Tables 1 and 2 show the clinical, histological, and imaging characteristics of the study patients before treatment of AH. At baseline, the MELD score, the ABIC score, and the histological findings of the liver biopsy specimen were not different between both groups, showing intermediate to high risk of death at 90 days. We found that the liver-to-spleen attenuation index was

Table 1. Clinical and histological characteristics of the study patients before treatment

	Improvers (n = 34)	Non-improvers (n = 24)	p value
Age (years)			
Mean (SD)	54.9 (8.1)	57.8 (5.3)	0.105*
Range	34.5–68.0	49.1–66.4	
Sex, n (%)			
Women	18 (53)	6 (25)	0.057†
Men	16 (47)	18 (75)	
Baseline MELD score			
Mean (SD)	19.1 (3.9)	19.0 (4.0)	0.942*
Range	13.0–27.0	13.0–28.0	
Baseline ABIC score			
Mean (SD)	8.3 ± 1.0	8.4 ± 0.7	0.636*
Range	6.9–9.9	6.5–10.3	
Degree of steatosis at biopsy (%)			
Mean (SD)	64.1 (25.1)	55.5 (25.4)	0.204*
Range	10.0–90.0	15.0–90.0	
Steatosis at pathology, n (%)			
≥30 %	28 (82)	17 (71)	0.300†
<30 %	6 (18)	7 (29)	
Liver injury score[a]			
Mean (SD)	6.2 (1.5)	5.8 (1.5)	0.263*
Range	3.0–8.0	4.0–8.0	
Hepatic venous pressure gradient (mm Hg)			
Mean (SD)	19.1 (2.8)	20.0 (2.3)	0.207*
Range	11.0–23.0	15.0–24.0	

ABIC age, serum bilirubin, INR and serum creatinine, *MELD* model for end-stage liver disease, *SD* standard deviation
* Student's t test; † Fisher's exact test; ‡ Pearson's χ^2 test
[a] Liver injury score, (1) degree of hepatocellular damage/ballooning (0, mild; 1, marked), (2) presence of Mallory bodies (0, absent; 1, present), (3) degree of polymorphonuclear (PMN) infiltration (0, mild; 1, moderate/severe), (4) degree of steatosis (0, <33 %; 1, 33 %–66 %; 2, >66 %), (5) lobular fibrosis (0, no fibrosis or zone 3; 1, fibrosis in zones 2 and 3; 2, pan- lobular fibrosis), (6) fibrosis stage (0, no fibrosis or portal fibrosis; 1, expansive periportal fibrosis; 2, bridging fibrosis and cirrhosis), (7) megamitochondria (0, no; 1, yes); and (8) presence and site of bilirubinostasis (0, none; 1, hepatocellular bilirubinostasis; 2, canalicular or ductular bilirubinostasis; 3, hepatocellular plus canalicular or ductular bilirubinostasis)

Table 2. Imaging characteristics of the study patients before treatment

	Improvers (n = 34)	Non-improvers (n = 24)	p value
Liver attenuation (HU)			
Mean (SD)	28.5 (19.3)	37.3 (11.2)	0.033*
Range	−36.6 to 47.3	−5.4 to 50.8	
Spleen attenuation (HU)			
Mean (SD)	38.7 (4.3)	40.1 (8.7)	0.483*
Range	25.8–47.0	31.0–70.7	
Liver-to-spleen attenuation index (HU/HU)			
Mean (SD)	0.73 (0.51)	0.96 (0.32)	0.043*
Range	−1.13 to 1.26	−0.15 to 1.28	
Steatosis at imaging, n (%)			
≥30 %	15 (44)	4 (17)	0.028†
<30 %	19 (56)	20 (83)	
Liver volume (cm^3)			
Mean (SD)	2460 (983)	1713 (660)	0.001*
Range	1085–6197	982–4067	
Spleen volume (cm^3)			
Mean (SD)	457 (230)	506 (249)	0.443*
Range	176–1116	137–1300	
Spleen-to-liver volume ratio (%)			
Mean (SD)	19.9 (9.6)	31.6 (14.9)	0.002*
Range	4.6–45.5	6.7–69.0	
Liver-to-body weight ratio (%)			
Mean (SD)	3.5 (1.2)	2.5 (1.0)	<0.001*
Range	1.3–6.8	1.4–5.5	
Spleen-to-body weight ratio (%)			
Mean (SD)	0.7 (0.3)	0.7 (0.4)	0.469*
Range	0.2–1.7	0.2–2.4	
Ascites, n (%)			
None	8 (24)	7 (29)	0.563‡
Moderate	8 (24)	3 (13)	
Severe	18 (52)	14 (58)	
Collaterals, n (%)			
None	8 (24)	0 (0)	0.026‡
Moderate	15 (44)	11 (46)	
Severe	11 (32)	13 (54)	

HU Hounsfield unit, *SD* standard deviation
* Student's t test; † Fisher's exact test; ‡ Pearson's χ^2 test

significantly higher in the non-improvers. However, this index was poorly correlated with the degree of steatosis ($r = -0.228$; $p = 0.085$) and the liver injury score ($r = -0.095$; $p = 0.477$). The liver-to-body weight (LBW) ratio of the improvers was significantly higher than that of the non-improvers ($p < 0.001$). Finally, portosystemic collaterals were always present and more important in the non-improvers, while the baseline hepatic venous pressure gradient and the grading of ascites were similar in both groups.

Table 3 shows the baseline anthropometric characteristics and body composition of the study patients. Body mass index and body surface area were similar in both groups. The adipose tissue index of the non-improvers was significantly lower than the improvers, which was mainly due to the difference between subcutaneous fat cross-sectional areas in respective group ($p = 0.027$). Although about three-quarters of the patients were sarcopenic at baseline, there was no statistically significant difference in skeletal muscle index between both groups.

At multivariate analysis, LBW ratio (OR, 3.73; 95 % CI, 1.65–8.46; $p = 0.002$) and subcutaneous fat (OR, 1.011; 95 % CI, 1.002–1.021; $p = 0.022$) retained independent prognostic value. The 2.4 % threshold for LBW ratio showed 88 % sensitivity, 63 % specificity, and an AUROC of 0.781 ± 0.064 ($p < 0.001$) for the identification of improvers. A 48 cm^2/m^2 threshold for subcutaneous fat index had 71 % sensitivity, 67 % specificity, and an AUROC of 0.658 ± 0.073 ($p = 0.043$) for the identification of improvers. The AUROC of the LBW ratio was significantly higher than that of subcutaneous fat index ($p = 0.004$). The combination of these two morphometric parameters was highly predictive of clinical response (Table 4), (Fig. 1).

At 6-month follow-up, eight patients (33 %) of the non-improvers group had died, while all patients of the improvers group were alive ($p < 0.001$). Patients dead at 6 months had higher initial ABIC score (8.8 ± 0.3 vs.

Table 3. Anthropometric characteristics and body composition of the study patients before treatment

	Improvers (n = 34)	Non-improvers (n = 24)	p value
Body mass index (kg/m^2)			
Mean (SD)	26.5 (5.1)	26.1 (4.2)	0.779*
Range	18.0–41.8	18.8–38.2	
Body surface area (m^2)			
Women	1.87 (0.23)	1.88 (0.16)	0.924*
Men	1.54–2.43	1.46–2.09	
Visceral fat cross-sectional area (cm^2)			
Mean (SD)	216 (63)	214 (60)	0.922*
Range	100–372	106–314	
Subcutaneous fat cross-sectional area (cm^2)			
Mean (SD)	213 (154)	146 (66)	0.027*
Range	78–834	33–353	
Total muscle cross-sectional area (cm^2)			
Mean (SD)	117 (33)	119 (33)	0.824*
Range	66–200	62–183	
Subcutaneous-to-visceral fat ratio (%)			
Mean (SD)	1.05 (0.77)	0.74 (0.39)	0.076*
Range	0.34–3.82	0.16–1.64	
Adipose tissue index (cm^2/m^2)			
Mean (SD)	151 (57)	126 (37)	0.047*
Range	79–311	71–249	
Skeletal muscle index (cm^2/m^2)			
Mean (SD)	40.7 (10.2)	40.9 (10.4)	0.947*
Range	24.2–68.6	22.6–58.4	
Sarcopenia, n (%)			
Yes	24 (71)	17 (71)	1.000†
No	10 (29)	7 (29)	

SD standard deviation
* Student's t test; † Fisher's exact test

8.3 ± 0.9; p = 0.016), lower LBW ratio (1.9 ± 0.3 % vs. 3.3 ± 1.2 %; p = 0.013), and showed clinical response less frequently (67 % vs. 100 %; p < 0.001). Other features, including age, MELD score, and subcutaneous fat index, were not significantly different. At multivariate analysis, only the LBW ratio was associated with survival (OR, 9.62; 95 % CI, 1.79–51.9; p = 0.008).

Validation cohort

The validation cohort included 42 patients (30 men; mean age, 53 years), with 27 improvers and 15 non-improvers. Patients from the validation set were comparable with those of the study set in terms of age, sex, weight, height, body mass index, body surface area, and MELD and ABIC scores.

The LBW ratio of improvers was significantly higher than that of non-improvers (mean 3.4 ± 0.2 % vs. 2.4 ± 0.3 %, p = 0.004). However, no difference could be observed between improvers and non-improvers when considering subcutaneous fat index (mean, 57 ± 5 cm^2/m^2 vs. 55 ± 8 cm^2/m^2; p = 0.58). The 2.4 % threshold value of the LBW ratio had 90 % sensitivity, 60 % specificity, 81 % positive predictive value, and 82 % negative predictive value for the identification of clinical response. At multivariate analysis, LBW ratio >2.4 % was the sole parameter that remained a significant predictor of clinical response (OR, 18.7; 95 % CI, 3.2–110.3; p = 0.001).

Discussion

AH is associated with significant rates of morbidity and mortality [3]. Several scoring systems (Maddrey discriminant function, MELD, ABIC, and Glasgow scores) have been introduced aiming at predicting early mortality and determining whether the patient could benefit from corticosteroids, a treatment that improves short-term survival [25]. These prognostic instruments are based on clinical and biological parameters, and have been validated in large clinical series [5]. The Lille score integrates the decrease in serum bilirubin at 7 days and helps to decide whether corticosteroid therapy should be stopped [26]. Histopathological findings on baseline liver biopsy help to grade the severity of ASH and thus provide additional information on clinical outcome [18]. However, this is an invasive procedure with potential complications in high-risk patients with disturbed coagulation and ascites [27]. Therefore, there is a justification to explore non-invasive methods, such as cross-sectional imaging.

The role of imaging in AH has been addressed by a limited number of clinical studies [28, 29]. To our best knowledge, none of these studies have shown that cross-sectional imaging may be used as a prognostic tool. Our results showed that the severity of liver disease and the nutritional status of patients with AH could be estimated with simple parameters derived from CT of the abdomen

Table 4. Response to treatment according to CT-based morphometric parameters

	n	Response		OR	95 % CI	p value
		Yes	No			
Liver volume-to-body weight ratio <2.4 % and subcutaneous fat cross-sectional area <48 cm^2/m^2	9	0 (0 %)	9 (100 %)	Reference	–	–
Liver volume-to-body weight ratio <2.4 % or subcutaneous fat cross-sectional area <48 cm^2/m^2	27	14 (52 %)	13 (48 %)	21	1–386	0.006
Liver volume-to-body weight ratio >2.4 % and subcutaneous fat cross-sectional area >48 cm^2/m^2	22	20 (91 %)	2 (9 %)	156	7–3575	<0.001

OR odds ratio, *95 % CI* 95 % confidence interval

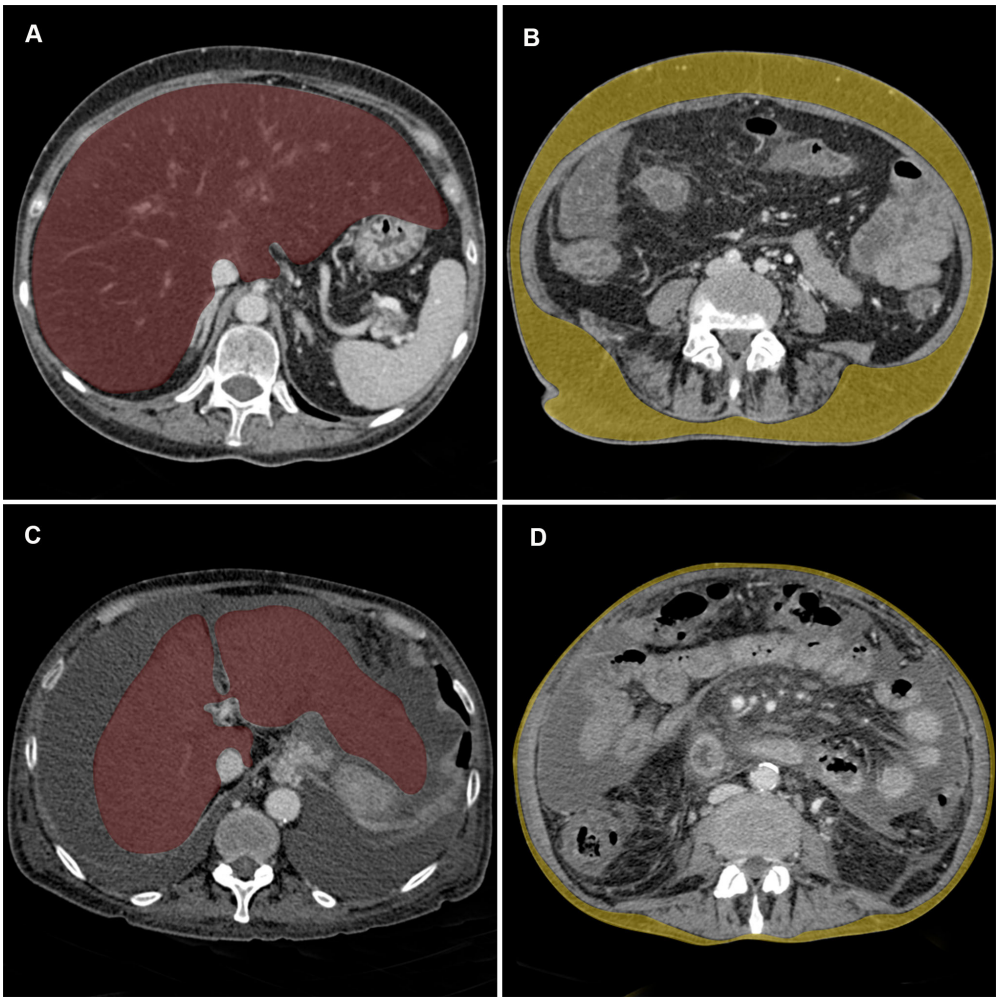

Fig. 1. Baseline computed tomography in decompensated liver disease. **A**, **B** A 55-year-old "improver" woman with a swollen and fatty liver (liver-to-body weight ratio, 6.8 %) and a large subcutaneous fat content (264 cm^2). **C**, **D** A 66-year-old "non-improver" man with a small and dysmorphic liver (liver-to-body weight ratio, 1.6 %) and a small subcutaneous fat content (66 cm^2). There are also a large amount of ascites and gastro-oesophageal varices.

that is often performed at hospital admission for diagnostic purposes. In the study group, we found that liver atrophy and subcutaneous fat content were independent factors that could predict a liver-related outcome at a relatively short term with a high specificity. In the validation cohort, the LBW ratio was also the sole predictive factor of clinical response. It is noteworthy that both baseline MELD and ABIC scores were not discriminant prognostic factors in both cohorts.

Alcoholic liver disease presents as a broad spectrum of disorders, ranging from simple fatty liver to end-stage cirrhosis, with superimposed episodes of ASH [3]. The gross morphology of the liver evolves according to the stage of liver disease. In liver steatosis, macroscopic fat infiltration of liver parenchyma is detected, and can be quantified by both CT and MRI [30]. Further progression toward inflammatory response with edema and appearance of sinusoidal hypertension lead to additional

congestion of the liver, resulting in hepatomegaly on palpation and diffuse liver enlargement on imaging [31]. Gradual hypotrophy of segment IV and posterior segments of the right liver and compensatory hypertrophy of segment I and left lobe lead to the typical dysmorphic appearance of the liver [32]. Finally, in advanced cirrhosis, the liver shows diffuse parenchymal atrophy with external contours abnormalities [33, 34]. Our hypothesis is that liver volume may reflect different stages of underlying liver disease. Thus, liver enlargement is related to an intermediate stage of the underlying cirrhosis with potential regenerative ability and response to treatment, provided that complete abstinence from alcohol has been achieved. Conversely, liver atrophy is related to more advanced stage disease, less efficient repair mechanisms, and diminished ability to restore liver function over time.

Malnutrition with vitamin deficit and reduced caloric intake is a common feature of chronic liver diseases that is often associated with a poor prognosis. Body composition analyses derived from imaging are widely employed in studies focusing on cancer, obesity, and diabetes. It has been shown in several clinical series that sarcopenia is independently associated with morbidity and mortality in patients with end-stage liver disease [35–37]. These studies included a large proportion of the patients with NASH or viral hepatitis, and none of these had specifically analyzed patients with AH. In our series, more than 70 % of the patients were considered as sarcopenic. A high prevalence of sarcopenia was also found in cirrhotic patients by Hanai et al. (68 %) and Giusto et al. (76 %) [37, 38].

We observed that reduced subcutaneous fat measured at baseline was a predictive factor of non-response to treatment. This finding is at first sight quite expected, as it goes along with general malnutrition consecutive to decreased food and vitamins intake characteristics of alcoholics. However, some recent work demonstrated that adipose tissue may not only reflect the nutritional status on an individual, but can also be biologically active [39]. Accordingly, adipocytes participate to the production of inflammatory mediators and may influence the severity of ASH [40]. However, this particular metabolic role seems to concern mostly visceral fat, which we did not identify as a predictor in our patients.

The strength of this study includes a large number of well-characterized patients with AH from a single center, who were evaluated early after hospital admission and carefully followed up during a 3-month period after decompensation. A detailed analysis of morphological parameters together with well-accepted clinic-biological scores of disease severity allowed us to identify liver volume as a key prognostic element both in the initial and validation cohorts. However, we acknowledge that our study suffers from some limitations, including the retrospective post hoc analysis of the data and the composition of our cohorts that limits our conclusion to patients with underlying cirrhosis. We did not use mortality as an end-point, but delta MELD as a valuable indicator of changes in liver function over time that is clinically relevant in decompensated cirrhosis [41]. The use of contrast-enhanced CT with multiphase acquisitions is associated with increasing risks of exposure to ionizing radiation and contrast-induced nephropathy. In addition, the cut-off values that we used for the definition of muscle mass depletion by CT have been derived from a population of obese patients affected by neoplasia, which might not represent normal values [24].

In conclusion, the liver volume appears to be a major positive prognostic factor in patients presenting with decompensated AH. It is easily extracted from a CT examination of the abdomen and could be integrated into existing scoring system, in order to better predict the short-term outcome. Furthermore, abdominal CT may also analyze body composition in a simple way, which could help to evaluate the degree of malnutrition and optimize renutrition strategies.

Compliance with ethical standard

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Appendix 2

Peribiliary Gland Dilatation in Cirrhosis: Relationship with Liver Failure and Stem Cell/Proliferation Markers

Summary:

Dilated peribiliary glands (PBG) in patients with cirrhosis are often an incidental finding although their significance and physiopathology remain unclear. We aimed to identify clinical factors associated with dilated PBG and to perform a detailed morphometric assessment of dilated PBG in cirrhotic patients undergoing liver transplantation. The methods used was that all consecutive cirrhotic patients undergoing LT at our institution between October 2006 and October 2011 were assessed for inclusion. Ten non-cirrhotic patients were included as controls. We performed morphometrical assessment of PBG, assessed baseline clinical factors associated with dilated PBG, immunohistochemistry staining with CK-19, MiB-1 and EpCAM, and radiological assessment of all available cases. The results showed that seventy-one patients met the inclusion criteria, 24% had PBG dilatation of >1000 μm. On multivariable analysis, MELD was the only significant factor associated with dilated PBG. Computed tomography and magnetic resonance imaging had high specificity but low sensitivity for the diagnosis of dilated PBG > 1000 μm (specificity 90-100%, sensitivity 25-29%). In conclusion, dilated PBGs are a common finding in explants of cirrhotic subjects undergoing LT and are associated with liver failure although diagnostic performance of cross-sectional imaging is inconstant. The high number of proliferative and EpCAM-positive cells lining the PBG may suggest a role of PBG in organ repair during liver failure.

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ORIGINAL ARTICLE

Peribiliary Gland Dilatation in Cirrhosis: Relationship with Liver Failure and Stem Cell/Proliferation Markers

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Abstract

Background and Aims Dilated peribiliary glands (PBG) in patients with cirrhosis are often an incidental finding although their significance and physiopathology remain unclear. We aimed to identify clinical factors associated with dilated PBG and to perform a detailed morphometric assessment of dilated PBG in cirrhotic patients undergoing liver transplantation (LT).

Methods All consecutive cirrhotic patients undergoing LT at our institution between October 2006 and October 2011 were assessed for inclusion. Ten non-cirrhotic patients were included as controls. We performed morphometrical assessment of PBG, assessed baseline clinical factors associated with dilated PBG, immunohistochemistry staining with CK-19, MiB-1 and EpCAM, and radiological assessment of all available cases.

Results Seventy-one patients met the inclusion criteria, 24% had PBG dilatation of >1000 µm. On multivariable analysis, MELD (OR 1.11 per unit increase in MELD, $p = 0.004$) was the only significant factor associated with dilated PBG. Compared to PBG < 1000 µm, large PBG had a higher proportion of EpCAM-positive (69 vs. 28%, $p < 0.001$) and MiB-1-positive lining cells (2.8 vs. 0.55%, $p = 0.036$). Computed tomography and magnetic resonance imaging had high specificity but low sensitivity for the diagnosis of dilated PBG > 1000 µm (specificity 90–100%, sensitivity 25–29%).

Conclusions Dilated PBGs are a common finding in explants of cirrhotic subjects undergoing LT and are associated with liver failure although diagnostic performance of cross-sectional imaging is inconstant. The high number of proliferative and EpCAM-positive cells lining the PBG may suggest a role of PBG in organ repair during liver failure.

Keywords Cirrhosis · Liver failure · Peribiliary glands · Stem cell · Abdominal imaging · Immunohistochemistry

Abbreviations

PBG	Peribiliary gland
MELD	Model for end-stage liver disease
MDCT	Multidetector computed tomography
MRI	Magnetic resonance imaging
MRCP	Magnetic resonance cholangiopancreatography
EpCAM	Epithelial cell adhesion molecule

Introduction

Dilated peribiliary glands (PBG) were first described in 1984 by *Nakanuma* et al. in a report of eight autopsy cases in patients with severe liver disease [1]. They are cystic structures, containing clear colorless fluid, adjacent to larger

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portal structures [2, 3]. On imaging, they are typically perihilar and they have a cystic (ranging from a few millimeters to centimeters) or tubular structure following portal tracts [4].

Clinically, dilated PBGs are often asymptomatic and are mostly considered an incidental finding; however, an increase in size of peribiliary cysts has been reported [4] and complications can sometimes ensue. Although rare, reported complications include compression of adjacent bile ducts, leading to jaundice, cholangitis, and, rarely, death [5, 6].

A systematic retrospective review of 1000 sequential liver autopsies showed that peribiliary cysts were found in only 5% of "normal" livers but in 40% of livers with hepatobiliary disease (primary or secondary liver malignancy, fatty liver, chronic hepatitis, and other histological abnormalities not reaching the stage of cirrhosis) and possibly a higher proportion in the limited subjects with cirrhosis [7]. However, this was a study based on autopsies, and the relevance of these findings in subjects with advanced liver disease is unclear. An imaging-based study found that 9% of livers with cirrhosis (defined clinically without liver biopsies in most cases) had signs on computed tomography compatible with dilated PBGs compared to 3% of non-cirrhotic controls [8]; however, this was not correlated with pathological findings.

Dilated PBGs are usually associated with preexisting liver disease. Although systematic clinical data are lacking, earlier case reports seemed to show that peribiliary cysts are associated with more severe liver disease and its complications (esophageal varices, portal vein thrombosis) [1, 6]. However, their pathogenesis remains unknown although it may involve ischemic changes or necroinflammatory activity [1, 7]. Recently, it has been suggested that PBGs harbor multipotent stem cells with the capacity to differentiate into cells of hepatocyte, cholangiocyte, and even pancreatic cell lineage [9, 10]. Cells in PBGs have been shown to proliferate and express stem and progenitor markers following bile duct injury [11, 12]; however, the changes in patients with advanced chronic liver disease have not yet been well characterized.

Thus, the aims of this study were to perform a detailed morphometric assessment of dilated PBG including histological characterization and immunohistochemistry for cellular proliferation and liver progenitor cell markers, as well as a radiological expression in a large population of patients with cirrhosis undergoing liver transplantation.

Patients and Methods

Patients

One hundred and eighty-four consecutive patients undergoing a liver transplantation at Geneva University Hospital between October 2006 and October 2011 were retrospectively assessed for inclusion. Exclusion criteria included pediatric patients, established biliary tract disorders, acute liver failure, retransplantation, and insufficient clinical data or histological material. Seventy-one patients were finally included in whom we collected clinical data at the time of transplantation. From the cohort of excluded patients, we selected 10 patients without cirrhosis or any diffuse liver or biliary disease to act as controls.

Morphometric Assessment

For all explanted livers, the right and left hepatic bile ducts at the level of the hepatic hilum were sampled. The presence of PBG was assessed at low (40×) and high (400×) magnification. As PBG dilatation is usually a diffuse process and not an isolated finding, we identified a representative PBG with the largest luminal diameter (with at least one other PBG of similar size to exclude isolated PBG dilatation). The maximal luminal diameter of PBG was measured and recorded using computerized morphometric analysis (AxioCam and AxioVision version 4.8.2.0 from Zeiss) with standard hematoxylin-eosin staining. PBG were judged to be small, medium-sized, or dilated based on luminal diameter (<200, 200–500, and >1000 µm diameter, respectively). If only an isolated dilated PBG was identified, only representative smaller PBG was used to assess PBG size. Clinical data from patients with dilated and non-dilated glands were compared.

Immunohistochemistry

Immuno-staining was performed using an automated system and reagents (Ventana Medical Systems, Tucson, AZ, USA). 5-µm-thick sections from formalin-fixed paraffin-embedded tissue blocks were incubated with mouse monoclonal human antibody MiB-1 against the proliferation marker Ki67 (Dako, M7240, 46 mg/L, 1:100 dilution), anti-CK19 (Dako, M0888, 20 mg/L, 1:50 dilution), anti-EpCam (Dako, M0804, 266 mg/L, 1:50 dilution), polyclonal rabbit antibody against CD133 (Biorbyt Ltd., 0.5 mg/ml, 1:100 dilution), or anti-Hep Par 1 (Dako, M7158, 80 mg/L, 1:400 dilution). Antigen retrieval was performed by heating slides in CC1 buffer (CC1; Tris-based buffer pH 8.4) for 12 min for CD133, 36 min for Ki67 and CK19, or 64 min for Hep Par 1. Concerning Epcam staining, antigenicity was retrieved by incubating slides 8 min in P1 protease buffer. Detection of primary antibodies was carried out using the amplified DabMap detection kit (HRP/SA complex using secondary biotinylated antibodies). Under high magnification (400×), the proportion of positive cells stained for EpCAM and MiB-1 was manually counted by two trained investigators in

multiple small glands (defined as <200 μm luminal diameter), one medium-sized gland (defined as 200–500 μm) and the largest dilated gland (defined as >1000 μm) if present. Results are given as the proportion of cells lining PBG staining for the marker, as reported [13].

Radiological Assessment

We reviewed available cross-sectional abdominal imaging (computed tomography or magnetic resonance imaging) of all included patients. We selected the imaging performed at the closest time before surgery. All examinations took place between October 2006 and September 2011. The images were anonymized and blindly evaluated separately by two experienced liver radiologists (RB and ST). The experts assessed the presence of peribiliary cysts, the size of the largest cyst, the presence of biliary dilatation on each liver lobe, portal vein thrombosis, and signs of chronic pancreatitis. For the computed tomography scans, the radiologist evaluated the presence of peribiliary cysts and biliary dilatation on the venous phase only. The images were displayed with an abdominal window setting (window level, 40; window width, 350), and the slices were reconstructed with a thickness of 2 or 3 mm. For the MRI scans, axial T2 sequences were chosen. All other sequences were excluded due to differences in MRI protocols used in abdominal imaging. The images were all single-shot non-triggered sequences (HASTE/SSH), and slice thickness was 5 mm.

Ethical Considerations

The study protocol was approved by the Institutional Review Board of the Hôpitaux Universitaires de Genève who allowed us to retrospectively analyze the clinical, biological, histological, and radiological data of the study population.

Statistical Analysis

All statistical tests were performed using the R statistical package version 3.1 (www.r-project.org). Continuous variables were presented as mean ± SD and compared using unpaired *t* test and Mann–Whitney *U* test as appropriate. Categorical variables were compared using the Chi-squared test or Fischer's exact test as appropriate. The relationship between dilated PBG and explanatory variables was adjusted for demographic and clinical factors using the bi-variate logistic regression model including all variables with a *p* value of ≤0.2 in univariable analysis. Statistical significance was set at a two-sided *p* value <0.05.

Results

Seventy-one out of 184 patients met the inclusion criteria. Patients were excluded for the following reasons: pediatric patients ($n = 20$), acute liver failure ($n = 9$), biliary tract

Table 1 Patient characteristics

Variables	Cases	Controls	*p* value
n	71	10	–
Age (years)	52.8 (±9.0)	36.5 (±15)	0.006
Male	55 (77%)	5 (50%)	0.12
Etiology of liver disease: alcohol	34 (48%)	0 (0%)	0.004
Etiology of liver disease: viral	40 (56%)	0 (0%)	0.001
PBG diameter (μm)	935 (±1250)	266 (±140)	<0.001
PBG dilatation >1000 μm	17 (24%)	0 (0%)	0.11
MELD	16.8 (±9.5)	–	–
Child-Pugh score	8.68 (±2.6)	–	–
HVPG (mmHg)	15.8 (±5.2)	–	–
Ascites	38 (54%)	–	–
HE	16/67 (24%)	–	–
AlkPhos (IU/l)	128 (±56)	–	–
Bilirubin (μmol/l)	118 (±180)	11.3 (±3.1)	<0.001
INR	1.54 (±0.77)	1 (±0)	<0.001

Continuous values compared with the independent samples *t* test and expressed as mean (±SD), categorical values compared with Fischer's Exact test and expressed as *n* (%). Normal upper values for AlkPhos and bilirubin are 102 IU/l and 25 μmol/l, respectively. *HVPG* hepatic venous pressure gradient, *HE* hepatic encephalopathy, *AlkPhos* alkaline phosphatase

disorders ($n = 11$), insufficient data ($n = 46$), and other reasons ($n = 11$). Baseline characteristics of the 71 patients and the 10 controls are shown in Table 1, as expected age, etiology of liver disease, and serum levels of bilirubin and INR were significantly different. All cases were patients with cirrhosis, and all controls were non-cirrhotic. Indications for liver transplantation in the 71 cases were chronic viral hepatitis ($n = 30$, 58%), alcohol abuse ($n = 33$, 47%), cryptogenic cirrhosis ($n = 3$, 4%), NASH ($n = 2$, 3%), and other ($n = 3$, 4%), whereas indications for liver transplantation in the 10 controls were malignant primary liver disease ($n = 2$, 20%), metastatic liver disease ($n = 3$, 30%), liver adenomatosis ($n = 2$, 20%), and other indications ($n = 2$, 20%).

Peribiliary Gland Dilatation Is Associated with an Increased MELD Score

All cases and controls had identifiable PBG in the hilar area. Seventeen out of 71 patients (24%) had PBG dilatation of >1000 μm (Fig. 1a, b, d) and in 20/71 of cases (28%) only small peribiliary glands <200 μm were present (Fig. 1c; Supplementary Table 1). On univariable analysis, patients with major PBG dilatation had more advanced liver disease as compared to those without this feature and were less likely to present with viral-induced liver disease (Table 2). On multivariable analysis including age, sex, MELD, alcohol, and viral etiology, MELD (OR 1.11 per unit increase in MELD, $p = 0.004$) was the only significant factor associated with dilated PBG (Table 2). Bilirubin and INR were not included in the multivariable analysis as they were multicollinear with MELD. PBG size was also significantly increased in cirrhotic cases compared to non-cirrhotic controls (934 vs. 266 μm, respectively, $p < 0.001$) with no control having PBG size over 1000 μm further suggesting that PBG dilatation is a reaction to liver insufficiency or liver disease (Table 1).

Due to the borderline association between alcohol liver disease and peribiliary glands, we assessed whether chronic extrahepatic biliary obstruction from chronic pancreatitis

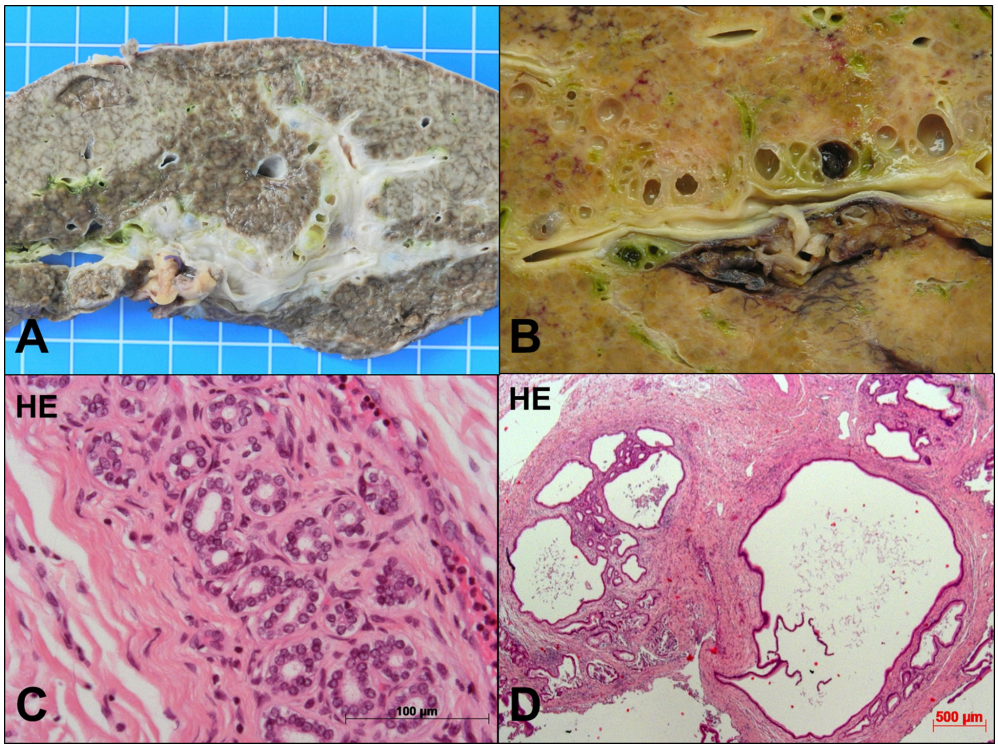

Fig. 1 Morphological and histological evaluation of PBG. **a**, **b** Macroscopical appearance of dilated PBG in cirrhotic explants (one *square* represents 1 cm). **c**, **d** Hematoxylin and eosin staining of small and dilated PBG (note difference in *scale*)

Table 2 Univariable and multivariable comparison of clinical characteristics associated with dilated PBGs

	No PBG dilatation	PBG dilatation	Univariable analysis	Multivariable analysis		
			p value	Adjusted OR	95% CI	*p* value
n	54 (76%)	17 (24%)				
Age (years)	52.2 (±9.8)	54.7 (±5.7)	0.20	1.02	0.945–1.13	0.55
Male sex	44 (81%)	11 (65%)	0.19	0.345	0.0600–1.88	0.21
Etiology: alcohol	23 (43%)	11 (69%)	0.089	2.26	0.488–11.7	0.30
Etiology: viral	35 (65%)	5 (31%)	0.023*	0.299	0.0562–1.40	0.13
MELD	14.9 (±8.5)	22.6 (±10)	0.011*	1.11	1.04–1.21	0.0039*
Ascites	27 (50%)	11 (65%)	0.40			
Alkaline phosphatase (IU/l)	131 (±58.7)	121 (±43.5)	0.485			
Bilirubin (μmol/l)	88.4 (±150)	212 (±220)	0.008*			
INR	1.38 (±0.53)	2.05 (±1.1)	0.028*			

Continuous values compared with the independent samples *t* test and expressed as mean (±SD), categorical values compared with Fischer's Exact test and expressed as *n* (%). Multivariable analysis performed by logistic regression for all variables with $p \leq 0.2$ in univariable analysis. Bilirubin and INR were not included in the multivariable analysis due to multicollinearity with MELD

OR odds ratio, *CI* confidence interval

* *p* value of <0.05

could be associated with PBG size. However, PBG dilatation was not associated with the presence of chronic pancreatitis, bile duct dilatation, or portal vein thrombosis as assessed by imaging (Supplementary Table 2). In addition, we did not identify morphological changes within PBG such as attenuated lining cells, mucinous metaplasia, or pancreatic acinar metaplasia and pancreatic acinar heterotopia.

Cells Lining Dilated PBG Display Increased Expression of MiB-1 and EpCAM

As PBG have been shown to harbor biliary tree stem/progenitor cells [14], and are believed to represent a reservoir for proliferating cells in response to injury [10, 12], we decided to assess the expression of CK19, EpCAM, CD133, and MiB-1 in cells lining PBGs by immunohistochemical studies. We assessed expression of EpCAM in 65/71 patients and of MiB-1 in 66/71 patients (Fig. 2). The proportion of cells staining for EpCAM within large PBGs was significantly increased compared to small PBGs (69 vs. 28%, respectively, $p < 0.001$) (Figs. 2a, b, and 3a). However, the proportion staining positively for EpCAM in small glands was not significantly different whether the sampled patient had overall dilated glands or non-dilated PBGs (27 vs. 31%, respectively, $p = 0.41$).

The proportion of lining cells staining positive for MiB-1 was also significantly increased in large PBG as compared to small PBG (2.8 vs. 0.55%, respectively, $p = 0.036$) (Figs. 2c, d, and 3b). CK-19 was not assessed as 100% of cells lining the glands stained positively for this antigen (Supplementary Figure 1). No PBG-lining cells stained positively for Hep Par 1 in six cirrhotic subjects with dilated glands greater than 1000 μm and three normal controls (Supplementary Figure 2). We further characterized PBG-lining cells in a subset of two cases by staining for CD133, a stem cell marker found to be associated with biliary tree stem/progenitor cells in PBG [10], and identified a subset of cells staining positive for CD133 (Supplementary Figure 3) although it must be highlighted that the sample size is limited and may not be representative of the overall population.

We next compared expression of EpCAM and MiB-1 in patients with cirrhosis and non-cirrhotic controls. The number of EpCAM-positive cells lining the PBGs was significantly higher in non-cirrhotic livers (57 vs. 28% in non-cirrhotic versus cirrhotic explants, respectively, $p = 0.012$), although there was no statistically significant difference for MiB-1 (5.2 vs. 5.5%, $p = 0.91$).

Cross-Sectional Imaging by CT Had High Specificity but Low Sensitivity to Identify PBG Dilatation

Cross-sectional imaging by multidetector computed tomography (MDCT) or magnetic resonance imaging (MRI) was performed prior to liver transplantation at a median time of 49 days (range 0–1073 days) and 251 days (range 8–1078 days), respectively. Sixty-four patients (90%) underwent at least one MDCT scan, 27 (38%) an MRI scan, and 29 (41%) had both examinations. Seven patients (10%) had no available imaging. Prevalence of pathologically documented dilated PBGs of >1000 μm in our population was 17/71 (24%), but MDCT identified only 9/64 patients (14%) as having dilated PBGs. When

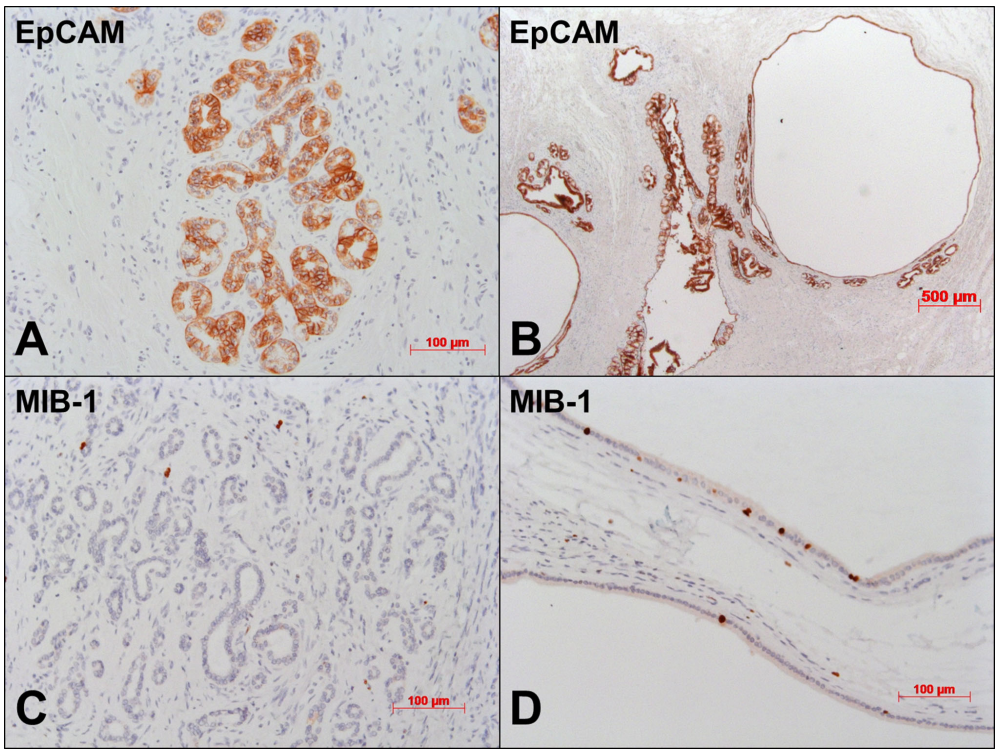

Fig. 2 Immunohistochemical evaluation of PBG. **a**–**d** EpCAM and MiB-1 staining of small (**a**, **c**) and dilated (**b**, **d**) PBGs. EpCAM, Epithelial cell adhesion molecule. *Scale* documented at *bottom right* of individual photomicrographs

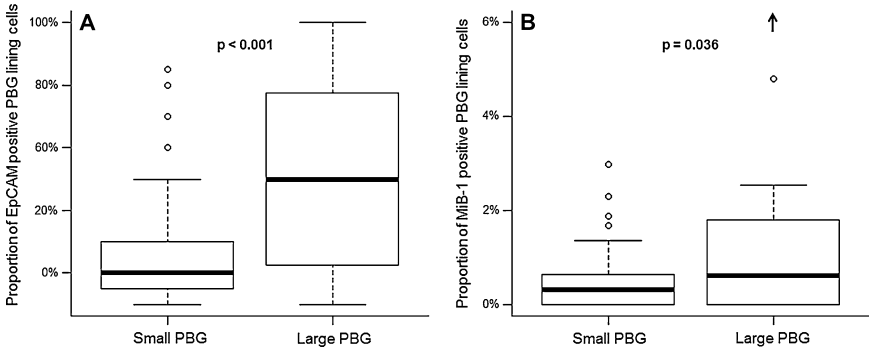

Fig. 3 Proportion of PBG-lining cells staining positive for EpCAM or MiB-1 in small and large PBGs. *Box plot* of cells staining positive for EpCAM (**a**) or for MiB-1 (**b**). In **b**, the *arrow* indicates three outliers in the large PBG group (11, 13, and 17%) that were not plotted to improve clarity

compared to pathological analysis, MDCT imaging had a specificity of 90% but a poor sensitivity of 29% to detect PBG dilatation of >1000 µm (Supplementary Table 3). This led to a positive predictive value (PPV) of 44% and negative predictive value (NPV) of 82%. Due to these results, we tested the diagnostic performance of MDCT when increasing the threshold of pathologically proven dilated PBGs to 3000 µm. After this correction, the prevalence of pathologically proven PBG dilatation decreased to 7/71 (9.9%). Diagnostic performance was mildly improved with a sensitivity of 40%, a specificity of 88%, a PPV of 22%, and a NPV of 95%.

MRI imaging identified only 1/27 patient with dilated PBG (4%), leading to a sensitivity of 25%, a specificity of 100%, a NPV of 89%, and a PPV of 100% for PBG > 1000 µm. For a histological threshold of PBG greater than 3000 µm sensitivity was 61% and NPV 99% (Supplementary Table 3).

Discussion

Although previous reports have documented the presence of peribiliary glands in subjects with liver disease, to our knowledge, this study is the first to pathologically and clinically define and characterize the presence of dilated PBG in subjects with advanced liver disease undergoing liver transplantation and correlating these findings with clinical markers of liver failure and cellular markers of regeneration and proliferative activity. We demonstrate that major dilatation of PBG is detected in 24% of our cohort, with the severity of liver failure being an independent factor associated with these dilated biliary structures. More importantly, we also show increased expression of EpCAM (a liver progenitor cell marker [15]) and MiB-1 (a marker of cell proliferation [16]) in cells lining these glands in large PBG as compared to small PBG. However, using a cut-off value of 1000 µm in diameter, imaging studies demonstrate a poor diagnostic performance, with a sensitivity of 29 and 25% using CT and MRI, respectively.

Peribiliary glands are structures that are located around large bile ducts predominant at the hepatic hilum. In our selected population of patients with advanced cirrhosis who received liver transplantation, major cystic dilatation of these glands was found in 24% of patients. As the prevalence of these dilated structures tends to increase with the severity of cirrhosis, the role of these PBG remains ill defined. Two hypotheses have been raised to explain the presence of these dilated PBG, including passive dilatation of the glands and pro-regenerative response to various stimuli. Indeed, it has been proposed that local alterations around large bile ducts in the region of the hepatic hilum induced by portal hypertension-associated circulatory changes [1], alcohol [17], lithiasis [11], ischemic, or inflammatory events may impair drainage of PBG into bile ducts [6]. As a consequence, passive dilatation ensues giving rise to dilated PBG which eventually may lead to mechanical obstruction of bile flow [18, 19]. A more recent hypothesis regarding the mechanisms associated with PBG dilatation relates to the biliary tree as a reservoir for multipotent stem cells [20, 21]. In particular, PBG have been identified as a niche for progenitor stem cells of endodermal origin which may express markers suggestive of bile duct, pancreas, and liver origin [9, 11]. Bile duct injuries appear as a major determinant of PBG dilatation, and injury to PBG have been recently incriminated as a major determinant of post-transplantation bile duct strictures due to insufficient biliary tissue repair [22]. In addition, in a recent report, PBG hyperplasia and metaplasia was observed in ducts with fibrosis in subjects with primary sclerosing cholangitis expressing Hedgehog pathway components and epithelial-to-mesenchymal transition traits suggesting a role in induction of biliary fibrosis and possibly in the association with cholangiocarcinoma [23]. However, hepatocellular insufficiency and portal hypertension in the absence of evident bile duct damage may also represent a stimulus for the emergence of these dilated PBG. Our data tend to support in part this statement, as major PBG dilatation at the level of the hepatic hilum is associated with an increased MELD score.

Sutton et al. reported an increased number of CK19+/MiB-1+ cells in the PBG of patients with severe damage to biliary epithelium as compared to patients with only mild disease [24]. In our patients, the diffuse positive staining for the cytokeratin marker CK19 confirms the epithelium immunophenotype of cells lining PBG [25]. The high density of cells with positivity for MiB-1 and EpCAM, a marker of both biliary and hepatocyte progenitor stem cells [20, 26], associated with CD133 staining, may possibly be seen as an effort to renew a damaged bile duct epithelium or to contribute to the pro-regenerative process in the setting of hepatocyte loss. The absence of Hep Par 1 staining suggests that the PBG-lining cells are not commited to a hepatocyte-like phenotype, although whether they are committed to a biliary, or even pancreatic tissue [10] regeneration cannot be answered based on our findings. Nevertheless, the description of an association between dilated PBG and high density of cells expressing certain markers suggesting a proliferating progenitor stem cell-like phenotype is novel and reinforces the view that multiple stem cells niches exist both in the liver (such as the canal of Hering and the ductular reaction) and in the biliary tree (the PBG) including the gallbladder [14], which may be activated following tissue injury to help contribute to organ regeneration and repair [12]. However, it must be highlighted that the sample size for CD133 staining in our

cohort was limited; therefore, additional studies should be undertaken to validate this point. In addition, more studies are needed to formally exclude other causes for PBG dilatation, such as passive dilatation of PBGs.

Both CT and MRI proved to be effective in the detection of peribiliary cysts in a small number of cases [4]. In our group of patients, however, these imaging techniques demonstrate a relatively poor diagnostic performance for pathologically proven dilated PBG. Using a morphological threshold of 1000 or 3000 μm, the sensitivity of MRI was 25 and 61%, respectively. Factors that may explain these findings include a limited number of patients with MRI (42%), our stringent criteria to diagnose morphologically dilated PBG, and the limited spatial resolution of MRI especially at the time of inclusion. In addition, with regards to considerable heterogeneity in the protocols used for imaging, we limited our analysis of 5-mm-thick standard axial sequences on MRI scans.

We acknowledge that our study suffers from some limitations, including the variable time interval between radiological studies and surgery and the application of a limited panel of antibodies used for immunohistochemistry.

In conclusion, in this large cohort of patients with advanced cirrhosis who received liver transplantation, dilated PBGs at the hepatic hilum diagnosed using stringent morphological criteria are found in 24% of cases, are inconstantly detected by computed tomography and MRI scans, and are associated with an increased MELD score. The increased number of proliferative cells with EpCAM stem cell marker positivity in the lining epithelium of the glands supports a contribution of PBG to organ repair in liver failure.

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Compliance with ethical standards

Conflict of interest None.

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Appendix 3

Ethylene vinyl alcohol copolymer for occlusion of specific portal branches during preoperative portal vein embolisation with n-butyl-cyanoacrylate.

Summary:

The objectives were to evaluate the safety and efficacy of ethylene vinyl alcohol copolymer (EVOH) injection for selective occlusion of portal branches considered at risk for non-target embolization during preoperative portal vein embolization (PVE). In the methods we included twenty-nine patients (mean age, 57 ± 17 years) submitted to PVE with n-butyl-cyanoacrylate (NBCA) and additional EVOH for selected portal branches were retrospectively analyzed. Degree of hypertrophy of the future liver remnant (FLR) and kinetic growth were assessed by CT volumetry performed before and 3-6 weeks after PVE. Clinical outcome and histopathological analysis of portal veins occluded with EVOH were reviewed. The results showed that indications for the use of EVOH were embolization of segment IV (n = 21), embolization of segmental portal branches with early bifurcation (n = 7) and PVE in a 1-year-old girl with cystic hamartomas. All targeted portal branches were successfully embolized. There were no cases with non-target embolization by EVOH. The degree of hypertrophy of the FLR was 14.3 ± 8.1% and the kinetic growth rate was 2.7 ± 1.8% per week. In conclusion EVOH is safe and effective for embolization of selected portal vein branches considered at risk for non-target embolization.

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INTERVENTIONAL

Ethylene vinyl alcohol copolymer for occlusion of specific portal branches during preoperative portal vein embolisation with *n*-butyl-cyanoacrylate

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Abstract

Objectives To evaluate the safety and efficacy of ethylene vinyl alcohol copolymer (EVOH) injection for selective occlusion of portal branches considered at risk for non-target embolisation during preoperative portal vein embolisation (PVE).

Methods Twenty-nine patients (mean age, 57 ± 17 years) submitted to PVE with *n*-butyl-cyanoacrylate (NBCA) and additional EVOH for selected portal branches were retrospectively analysed. Indications for the use of EVOH and the selected portal branches were evaluated. Degree of hypertrophy of the future liver remnant (FLR) and kinetic growth were assessed by CT volumetry performed before and 3–6 weeks after PVE. Clinical outcome and histopathological analysis of portal veins occluded with EVOH were reviewed.

Results EVOH was indicated intraoperatively for embolisation of selected portal branches that the operator reported at risk to provoke non-target embolisation with NBCA. Indications for the use of EVOH were embolisation of segment IV (n = 21), embolisation of segmental portal branches with early bifurcation (n = 7) and PVE in a 1-year-old girl with cystic hamartomas. All targeted portal branches were successfully embolised. There were no cases with non-target embolisation by EVOH. The degree of hypertrophy of the FLR was 14.3 ± 8.1% and the kinetic growth rate was 2.7 ± 1.8% per week.

Conclusion EVOH is safe and effective for embolisation of selected portal vein branches considered at risk for non-target embolisation.

Key Points

- *EVOH is another effective liquid embolic agent for preoperative PVE.*
- *EVOH is relatively simple to handle with a minimal risk of non-target embolisation.*
- *During PVE, some portal branches considered complicated to occlude with NBCA may be efficiently embolised with EVOH.*

Keywords Portal vein · Embolisation · Therapeutic · Ethylene vinyl alcohol copolymer · Hepatectomy

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Abbreviations

ALT	Alanine transaminase
AST	Aspartate transaminase
CT	Computed tomography
DMSO	Dimethyl sulfoxide
EVOH	Ethylene vinyl alcohol
FLR	Future liver remnant
NBCA	*n*-butyl cyanoacrylate
PVE	Portal vein embolisation

Introduction

Preoperative portal vein embolisation (PVE) is a well-established method to stimulate hypertrophy of the future liver remnant (FLR) by means of growth factor production and redirection of portal blood flow towards the FLR, thereby enhancing the safety of extensive liver resection [1, 2]. Numerous embolic agents have been used for PVE to achieve the occlusion of the targeted portal branches, i.e. gelatin sponge, different types and size of beads, polidocanol foam, Ethibloc, ethanol, fibrin glue, *n*-butyl-cyanoacrylate (NBCA), or combinations of these materials with coils or nitinol plugs [3]. A mixture of NBCA and iodised oil is frequently used. It leads to important periportal inflammation and a persistent occlusion of the portal branches, preventing recanalisation [4]. Therefore, this embolic agent seems to have a greater effect on FLR hypertrophy, as confirmed by two retrospective clinical series [5, 6]. Nevertheless, the use of NBCA may be difficult and technically demanding. Polymerisation of this adhesive agent depends on dilution with lipiodised oil and rate of injection and small pieces of polymerised glue may migrate to non-target portal branches. The risk of undesirable migration of NCBA may be particularly higher at the final moments of the procedure when the operator has to occlude the proximal portion of target branches and when the overall portal venous flow changes because of branch occlusion. Furthermore, small-sized fragments of glue residue may have remained inside or adhered at the distal tip of the catheter during the procedure.

Ethylene vinyl alcohol copolymer (EVOH; Onyx, ev3) is a cohesive liquid embolic agent with low viscosity that consists of ethylene vinyl alcohol dissolved in dimethyl sulfoxide (DMSO) and suspended micronised tantalum powder. This product is used in interventional neuroradiology for treatment of cerebral arteriovenous malformations or dural fistula, because it can be delivered with very accurate control, avoiding unplanned occlusions and unintentional reflux [7]. Recently, EVOH has also been reported as a promising alternative embolic material for peripheral applications, such as embolisation of acute haemorrhage, or type I and II endoleaks [8, 9]. The aim of this study was to evaluate safety and efficacy of the use of EVOH for selective portal branch occlusion during preoperative PVE.

Materials and methods

Subjects

From December 2010 to January 2015, a total of 110 PVE procedures were performed in our institution. The present clinical study is a retrospective analysis of a group of 29 patients who underwent PVE with NBCA–Lipiodol and additional EVOH, without any other embolic materials. The institutional review board gave a waiver for patient informed consent (CE-12-193R). The indication for preoperative PVE in these patients was the need for major liver resection for hepatic malignancy with an FLR ratio less than 25% in patients without underlying liver disease or less than 40% in patients with chronic liver disease. The baseline characteristics of the study population are shown in Tables 1 and 2.

Portal vein embolisation

PVE was performed by three interventional radiologists with 7, 12 and 15 years of experience in hepatobiliary procedures. A prophylactic dose of 2 g of ceftriaxone was intravenously administered 1 h before the procedure. Under general anaesthesia ($n = 27$; 93%) or conscious sedation ($n = 2$; 7%), access to the portal vein was obtained in all cases with a contralateral ($n = 23$; 79%) or ipsilateral ($n = 6$; 21%) approach. A portal vein branch was punctured with a 21-G Chiba needle via a percutaneous transhepatic route under sonographic guidance. A 5-F introducer sheath was inserted in the main portal vein with the Seldinger technique. A direct portography was obtained in the oblique anterior projection and the baseline portal pressure was measured. The targeted segmental portal branches were selectively catheterised one by one with a 5-F Multipurpose or Simmons-2 catheter (Cook). The embolisation was carried out under fluoroscopic guidance with a mixture of NBCA (Histoacryl; B. Braun) and iodised oil (Lipiodol; Guerbet) at a ratio of 1:1 to 1:4. The catheter was regularly flushed with a 20% glucose solution between each injection. When embolisation with EVOH was indicated (portal branches of segment IV, portal branches with early bifurcation in segment V, VI and VIII), the distal portion of the portal branch was selectively catheterised with a DMSO-compatible 2.4-F microcatheter (Rebar 18, eV3). The microcatheter hub was flushed with 10 mL saline and its dead space was filled with 0.5 mL of DMSO. EVOH was injected into the microcatheter with thumb pressure in aliquots of 1 mL, at a maximum rate of around 0.3 mL/min, and under continuous fluoroscopic control. Whenever EVOH reflux occurred, the microcatheter was retracted to a more proximal position. Upon completion of portal branch occlusion, EVOH injection was stopped, slight aspiration was applied for a few seconds, and the microcatheter was gently pulled back.

At the end of the PVE procedure, a final portography was obtained, as well as a final measurement of the portal pressure. The puncture track was systematically embolised. Technical success of PVE was defined as a complete occlusion of the portal branches feeding the liver to be resected [10]. Reported indications for the use of EVOH, amount of contrast medium, mean dose area product and duration of procedure were evaluated.

Table 1 Baseline characteristics of patient population ($N = 29$)

Demographics data		
Age [years]	57±17	(1-85)
Male gender	18	(62.1%)
Body mass index [kg/m^2]	23.3±3.7	(16.0-29.7)
Body surface area [m^2]	1.75±0.34	(0.39-2.12)
Primary tumour		
Colorectal adenocarcinoma	12	(41.4%)
Cholangiocellular carcinoma	8	(27.6%)
Hepatocellular carcinoma	6	(20.7%)
Invasive ductal carcinoma of the breast	1	(3.4%)
Haemangioendothelioma	1	(3.4%)
Cystic mesenchymal tumour	1	(3.4%)
Underlying liver disease		
Child A cirrhosis	6	(20.7%)
Chronic biliary obstruction	3	(10.3%)
Sinusoidal obstruction syndrome	2	(6.9%)

Continuous values are expressed as mean ± standard deviation and range in parentheses. Categorical data are expressed as frequency and percentage in parentheses

Follow-up

All patients were observed at least overnight and serum liver tests were repeated before discharge. Adverse events were reported according to the Society of Interventional Radiology classification of complications [11]. All patients were examined with contrast-enhanced computed tomography (CT) of the abdomen during initial workup. CT images (standard filtered back-projection algorithm; slice thickness, 1.5 mm; slice interval, 1.0 mm) were obtained during the portal phase after intravenous injection of contrast media (iodine content, 350 mg I/mL; volume, 2 mL/kg body weight; flow rate, 4 mL/s) with a 64-detector row CT scanner. CT images were processed using open-source software (OsiriX 64-bit version 4.3). The FLR was manually contoured, according to the resection plane and excluding the large hepatic vessels, the major fissures and any tumour. The FLR volume was calculated in cubic centimetres and the FLR ratio was defined as the ratio of FLR volume to the estimated total liver volume, using the body surface area [12].

Three to six weeks after PVE procedure, FLR volume was reassessed on a contrast-enhanced CT of the abdomen. PVE was considered clinically successful when the FLR ratio became greater than 25% in patients without liver disease or greater than 40% in patients with chronic liver disease. The indication for major hepatectomy was rediscussed during a multidisciplinary meeting. The cause of cancellation was recorded and the resection rate was calculated accordingly. The histopathology report of the resected specimen was reviewed.

Statistical analysis

Degree of hypertrophy was defined as the difference between FLR ratio before and after PVE and the kinetic growth rate was defined as the degree of hypertrophy per week after PVE [13]. Continuous values were expressed as mean (standard deviation) and range. Categorical data were expressed as frequency (percentage). A p value of less than 0.05 was considered as statistically significant. Statistical analysis was done using SSPS 15.0 statistical package (SPSS Inc, Chicago, IL, USA).

Results

Portal vein embolisation

The manometric and biological changes after PVE are shown in Table 2. Standard PVE was performed with injection of 5.9 ± 2.1 mL (3.0–10.5 mL) of the NBCA–Lipiodol mixture in

Table 2 Volumetric, manometric and biological changes after portal vein embolisation

	Before PVE	After PVE	p
Volumetric and manometric data			
FLR volume (cm^3)	397 ± 130 (104–596)	611 ± 181 (216–1019)	< 0.0001
FLR ratio (%)	28 ± 7 (10–39)	42 ± 11 (22–67)	< 0.0001
Mean portal pressure (mmHg)	7 ± 4 (3–10)	9 ± 5 (3–14)	0.702
Liver function tests			
ALT < 50 U/L	60 ± 45 (13–173)	128 ± 107 (19–460)	0.040
AST < 50 U/L	56 ± 68 (12–389)	172 ± 212 (16–907)	0.028
Total bilirubin < 25 μmol/L	18 ± 8 (8–131)	19 ± 7 (8–28)	0.691
INR < 1.5	1.1 ± 0.2 (1.0–1.4)	1.1 ± 0.2 (1.0–1.5)	0.878

Values are expressed as mean ± standard deviation and range in parentheses

ALT alanine transaminase, *AST* aspartate transaminase, *FLR* future liver remnant, *INR* international normalised ratio, *PVE* portal vein embolisation

the right portal vein ($n = 21$; 72%), left portal vein ($n = 2$; 7%) or segmental branches in both hepatic lobes ($n = 3$; 10%). During the same session, percutaneous biliary drainage of the FLR was performed in three patients with biliary obstruction, while radiofrequency ablation was carried out in two patients with metastases in the FLR [14].

The main indication for the use of EVOH was additional embolisation of segment IV before resection extending to this segment in 21 patients (72%). Two to five portal branches of segment IV were completely occluded (Fig. 1). The other indications were embolisation of portal branches with early bifurcation in segment V, VI and VIII ($n = 7$; 24%) and preoperative PVE in a 14-month-old toddler with cystic mesenchymal hamartoma (3%) [15]. In two patients (7%), complex embolisation of segments II, III, IV, V and VIII required a bilateral PVE with a special technique (Fig. 2). The mean volume of injected EVOH was 2.9 ± 2.0 mL (1.0–8.0 mL).

Technical success was achieved in all procedures (100%). The puncture track was embolised with gelatin sponge (Gelfoam; Pharmacia Upjohn) ($n = 26$; 90%), EVOH ($n = 2$; 7%) or NBCA–Lipiodol ($n = 1$; 3%). The total amount of iodixanol 270 mg I/mL (Visipaque; GE Healthcare) injected during the procedure was 272 ± 115 mL (30–450 mL), and the mean dose area product was 216 ± 163 Gy • cm^2 (11–872 Gy • cm^2). Overall, the mean duration of the procedure was 83 ± 36 min (38–146 min).

Follow-up

The follow-up after portal vein embolisation is summarised in Table 3. No major complication related to the PVE procedure was observed. Seventeen patients (59%) were discharged within 24 h of the PVE procedure, and nine patients (31%) within 48 h. The three patients (10%) with combined biliary drainage were discharged 3–9 days afterwards. The mean interval time between PVE and follow-up CT was 32 ± 8 days (16–70 days). The volumetric changes after PVE are shown in Table 2. The degree of hypertrophy of the FLR was 14.3 ± 8.1% (1.2–35.0%) and the kinetic growth rate was 2.7 ± 1.8% per week (0.2–15.3% per week).

Ultimately, 25 patients underwent curative liver resection and four patients were excluded from it, corresponding to an overall resection rate of 86%. The mean interval time between PVE and hepatectomy was 47 ± 17 days (24–87 days). Among the 25 patients who underwent surgery, two patients (6.9%) had grade A postoperative liver failure at day 5, according to International Study Group of Liver Surgery (ISGLS) classification, specific morbidity was limited to Clavien–Dindo grade III complications and there was no mortality at 3 months [16, 17]. Histopathological assessment of EVOH was found in nine pathology reports (31%). The embolised portal vessels were occluded by blackish materials, without sign of recanalisation, and some hepatic lobular sinusoids were partially filled with EVOH. The venous wall was partially destroyed and surrounded by giant cell reaction of the foreign body type (Fig. 3).

Discussion

In the present study, PVE with NBCA–Lipiodol and additional EVOH was always technically successful with optimal filling of all targeted portal vessels. We did not observed any non-target embolisation of EVOH in the FLR, nor adverse effects related to the toxicity of DMSO. Only one patient with a Klatskin tumour presented with a major complication related to suboptimal biliary drainage, but not to PVE itself. After PVE, the degree of FLR hypertrophy significantly increased and was within the benchmark suggested by van Lieden et al. and the Cardiovascular and Interventional Radiological Society of Europe (CIRSE) guidelines for PVE [3, 10]. Moreover, the kinetic growth rate was above the threshold of 2% per week, which is the best predictor of favourable surgical outcomes after major hepatic resection [13]. Liver resection was possible in the vast majority of patients and the resection rate was also consistent with the recommended guidelines. The reasons for non-resection were mainly related to disease progression.

Percutaneous PVE is the most widely accepted technique for preoperative induction of FLR hypertrophy in patients requiring extensive hepatectomy with inadequate FLR volume, despite the emergence of new surgical or interventional procedures, such as associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) or simultaneous transhepatic portal and hepatic vein embolisation (liver venous deprivation technique) [18, 19]. The PVE procedure is generally considered technically demanding, requiring interventional radiologists with experience in hepatobiliary interventions. Challenges of PVE include that (1) selective catheterisation and navigation in the different portal branches can be difficult on fluoroscopy as their images may be superimposed on each other; (2) the capacity of the vascular bed is large, requiring a great amount of embolic agent to obtain a complete embolisation; (3) NBCA polymerises within seconds, which could lead to proximal occlusion of target portal vein or inadvertent embolisation of non-targeted portal branches in the FLR, and could potentially jeopardise subsequent resection [20, 21]; (4) the delivery catheter often occludes or can be trapped in the very adhesive NBCA agglomerate. These drawbacks could be reduced by increasing the ratio mixture with Lipiodol, by using a proximal vascular plug and by frequent catheter flush with a non-ionic solution [5, 6, 20, 22]. However, many authors acknowledge the technical limitations of NBCA, especially

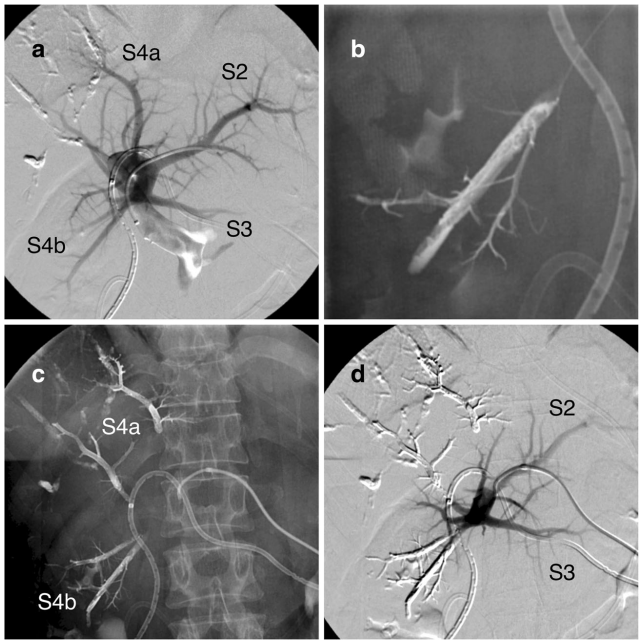

Fig. 1 Portal vein embolisation of segment IV in a 52-year-old woman with a Klatskin tumour in the right liver (Bismuth 3R). **a** Portography after right portal vein embolisation with NBCA glue showing the portal branches of the left hepatic lobe (S2, S3) and segment IV (S4a, S4b). **b** Selective injection of EVOH through a 2.4-F microcatheter in a portal branch of segment S4b. **c** Fluoroscopic image after selective embolisation of four portal branches in segment IV. **d** Final portography showing complete occlusion of all portal branches except segments II and III

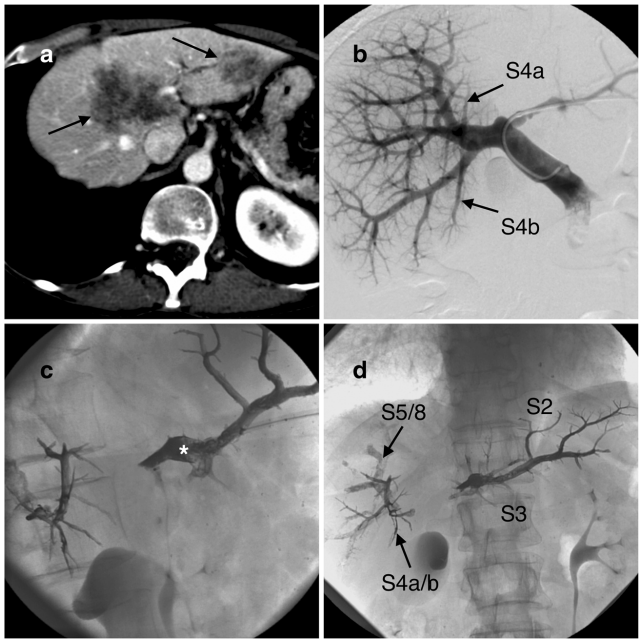

Fig. 2 Portal vein embolisation tailored to prepare left hepatectomy extended to right anterior sector in a 62-year-old woman with hepatic metastases of colorectal origin. **a** Contrast-enhanced CT shows two large masses (black arrows) in the left hepatic lobe and segments IV and VIII. **b** Portography was obtained by an ipsilateral approach in the left portal vein. Two portal branches for segment IV (S4a, S4b) are coming from the right portal vein. **c** After microcatheter retraction in the left portal vein (white asterisk), EVOH solidifies proximally and, after 3 min, flows backwards in the left hepatic lobe. **d** Fluoroscopic image at the end of the procedure showing NBCA glue in the portal branches of the right anterior sector (S5/8) and complete occlusion of portal branches in segment IV (S4a/b) and in left hepatic lobe (S2, S3)

Table 3 Follow-up after portal vein embolisation

Major complications		
Acute cholangitis	1	(3.4%)
Minor complications		
Transient fever and pain	3	(10.3%)
Non-target embolisation of NBCA	2	(6.9%)
Non-target embolisation of EVOH	0	(0%)
Type of hepatic surgery		
Extended right hepatectomy	21	(72.4%)
Extended left hepatectomy	2	(6.9%)
Complex hepatic resection	2	(6.9%)
Cancellation of hepatic surgery		
Tumour progression on CT	2	(6.9%)
Peritoneal metastases at laparotomy	1	(3.4%)
Inadequate FLR growth	1	(3.4%)

Data are expressed as frequency and percentage in parentheses

CT computed tomography, *EVOH* ethylene vinyl ethanol, *FLR* future liver remnant, *NBCA* n-butyl cyanoacrylate

when dealing with the portal branches of segment IV, in the presence of portal bifurcation variants, or when the anatomy and flow dynamics of the portal vein are modified by large tumours or chronic liver disease [23–27]. Other embolic agents have been used for PVE, but they all have their own limitations (Table 4) [28–32]. Onyx is an embolisation agent consisting of EVOH copolymer, which remains liquid, as long as it is saturated with DMSO solvent. The major advantages of EVOH compared to the cyanoacrylates are its non-adherence, cohesiveness and high vascular penetration [33]. Therefore, we thought EVOH might be a good candidate agent for PVE in high-risk situations, when NBCA glue was contraindicated. In our institution, when more extended or atypical resections are planned, we tailor the PVE procedure by including all future resected segments, as already described by De Baere et al. [34]. Thus, the main indications for EVOH in our series were embolisation of several portal branches in segment IV or early bifurcation of a segmental vein from the right portal branch. In the young child, the preoperative PVE was completely achieved with EVOH to avoid non-target embolisation in a very small liver. In most cases, the distal placement of the microcatheter, the high fluoroscopic visibility of EVOH and the injection under slow controlled pressure resulted in a complete filling of all targeted portal vessels with good monitoring of the progression of the substance. During all embolisations with EVOH, we did not observed either obstruction of the microcatheter or entrapment of its distal tip. Also, the two cases of non-target embolisation in the FLR were caused by NBCA and not by EVOH. Histopathological analysis showed complete filling of the portal vessels with EVOH and deep penetration to the sinusoidal level. There was also a significant inflammatory reaction of the periportal tissue, which is considered to be a favourable factor for contralateral liver regeneration [4]. These findings were already described by Smits et al. after trans-sinusoidal wedged injection of EVOH in pig livers [35].

The limitations of this study include the following. First, this is a retrospective study with a relatively small and heterogeneous sample size, which limits statistical power. Second, we did not perform the entire PVE procedure with EVOH, because of the volume of liquid required and the high cost of EVOH. Thus, a comparison of clinical outcomes after PVE between EVOH and another embolic material was not possible. Third, portal embolisation with EVOH requires a slight increase in procedure time and delivered radiation dose, because its injection is done slowly and followed by continuous fluoroscopic control. However, assuming that EVOH could assist in complicated cases, procedure time might be compensated for. In any case, these hypotheses need a study with a control group to be evaluated. Finally, the details of histopathological analysis were available only for a minority of patients, not allowing a robust assessment of the effects induced by EVOH on the vessels and surrounding tissue.

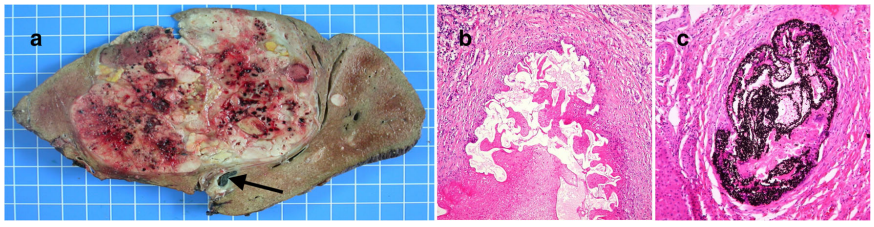

Fig. 3 Histopathological findings after portal vein embolisation with NBCA glue and additional EVOH in an 80-year-old man with hepatocellular carcinoma. **a** Liver specimen after right lobectomy showing EVOH as blackish material occluding a portal branch of segment IV (black arrow). Photomicrographs (haematoxylin and eosin staining, original magnification ×25) of the embolised liver showing portal vessels occluded by either NBCA glue (**b**) or EVOH (**c**)

Table 4 Characteristics of different embolisation agents for portal vein embolisation

Embolic agent	Delivery control	Radio-opacity	Vessel occlusion	Inflammatory reaction	Price
NBCA–Lipiodol	Needs high level of experience	High	Subcomplete, permanent	Major	Cheap
Microparticles + coils	Good	Very high (coils)	Subcomplete, possible recanalisation	Minor	Expensive
Gelatine sponge	Intermediate	No	High rate of recanalisation	No	Cheap
Fibrin glue	Requires occlusion balloon	Intermediate	High rate of recanalisation	Minor	Intermediate
Ethanol	Requires occlusion balloon	No	Complete, permanent	Major	Cheap
Sclerosing foams	Requires occlusion balloon	Requires contrast media	Possible recanalisation	Intermediate	Cheap
EVOH copolymer	Very good	Very high	Complete, permanent	Major	Very expensive

EVOH ethylene vinyl ethanol, *NBCA* *n*-butyl-cyanoacrylate

In conclusion, on the basis of the available data, PVE with complementary EVOH was feasible, safe and effective before major liver resection. Since this liquid embolisation agent is relatively simple to handle with a minimal risk of non-target embolisation, it may be helpful for occluding portal branches, which are not accessible with conventional embolic materials. Currently, EVOH provides valuable assistance on an individual case basis; but in view of its current cost, it cannot be used routinely.

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Compliance with ethical standards

Guarantor The scientific guarantor of this publication is Professor Sylvain Terraz.

Conflict of interest The authors of this manuscript declare no relationships with any companies whose products or services may be related to the subject matter of the article.

Statistics and biometry No complex statistical methods were necessary for this paper.

Informed consent Written informed consent was waived by the institutional review board.

Ethical approval Institutional review board approval was obtained.

Methodology
- retrospective
- observational
- performed at one institution

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Appendix 4

Endovascular treatment of arterial complication after liver transplantation: long-term follow-up evaluated on Doppler ultrasound and magnetic resonance cholangiopancreatography

Summary:

The purpose was to evaluate long-term arterial patency and abnormalities of bile ducts in patients that had endovascular treatment for arterial complications after liver transplantation (LT). The methods were that between 2004 and 2014, 1048 LTs were consecutively performed in our institution and 53 patients were diagnosed and treated by endovascular techniques for arterial complications such as stenosis or thrombosis. Radiological and surgical data were retrospectively analyzed, and survivors were contacted to undergo follow-up Doppler ultrasound (DUS) of the HA and magnetic resonance cholangiopancreatography. The primary technical success of endovascular treatment was 94% (n=50). The patency rate of HA at 5-year was 81%. After a median follow-up of 58 months, 17 patients (32%) developed radiological features of ischemic cholangiopathy (IC), including 7 patients with abnormal DUS and 10 with normal DUS. Patients who presented with complications of the HA in the first 3 months after LT developed IC more frequently (42%) than others (12%) (p=0.028). No other factor was associated with the development of IC. In conclusion IC was more often observed when HA complication occurred within the first 3 months after LT. The presence of IC was not excluded by a normal DUS during follow-up.

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Endovascular Treatment of Arterial Complications After Liver Transplantation: Long-Term Follow-Up Evaluated on Doppler Ultrasound and Magnetic Resonance Cholangiopancreatography

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Abstract

Purpose To evaluate long-term arterial patency and abnormalities of bile ducts in patients that had endovascular treatment for arterial complications after liver transplantation (LT).

Materials and Methods Between 2004 and 2014, 1048 LTs were consecutively performed in our institution and 53 patients (42 men; age range 19–69) were diagnosed and treated by endovascular techniques for arterial complications such as stenosis, thrombosis, dissection or kinking of the hepatic artery (HA). Radiological and surgical data were retrospectively analyzed, and survivors were contacted to undergo follow-up Doppler ultrasound (DUS) of the HA and magnetic resonance cholangiopancreatography.

Results The primary technical success of endovascular treatment was 94% (n = 50). The patency rate of HA at 5-year was 81%. After a median follow-up of 58 months, 17 patients (32%) developed radiological features of ischemic cholangiopathy (IC), including 7 patients with abnormal DUS and 10 with normal DUS. Patients who presented with complications of the HA in the first 3 months after LT developed IC more frequently (42%) than others (12%) (p = 0.028). No other factor was associated with the development of IC.

Conclusion IC was more often observed when HA complication occurred within the first 3 months after LT. The presence of IC was not excluded by a normal DUS during follow-up.

Keywords Liver transplantation · Hepatic artery · Endovascular procedures · Ischemic cholangiopathy

Abbreviations

CDT	Catheter-directed thrombolysis
CI	Confidence interval
DUS	Doppler ultrasound
HA	Hepatic artery
IC	Ischemic cholangiopathy
MDCT/A	Multidetector-computed tomography/ arteriography
MRCP	Magnetic resonance cholangiopancreatography
LT	Liver transplantation
PTA	Percutaneous transluminal angioplasty
PACS	Picture archiving and communication system

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Implications for Patient Care

1. The only risk factor of ischemic cholangiopathy development after liver transplantation is the early occurrence of arterial complication even if the endovascular treatment was initially successful.
2. Ischemic cholangiopathy cannot be excluded by a normal Doppler ultrasound exam during follow-up after liver transplantation.
3. In patients with abnormal Doppler ultrasound during follow-up after liver transplantation, only the half developed ischemic cholangiopathy.

Summary Statement

If the arterial complication occurred within the first three months after transplantation, the risk of ischemic cholangiopathy is 42%, whatever the presence or not of normal Doppler ultrasound.

Introduction

Biliary complications are important causes of morbidity and mortality after liver transplantation (LT), with an incidence of 10–30% [1–4]. Ischemic cholangiopathy (IC) can severely damage the biliary tree and the management of these lesions can be difficult, often extending over many years or requiring re-transplantation. Hepatic artery (HA) thrombosis represents a major cause of IC [3, 5]. Risk factors for the development of HA thrombosis such as anastomotic stenosis [6] are actively searched for by Doppler ultrasound (DUS), immediately after transplantation as well as during the lifetime of transplanted patients. Multidetector-computed tomography arteriography (MDCTA) is performed to confirm the diagnosis when stenosis or thrombosis is suspected on DUS and endovascular treatments, such as percutaneous angioplasty (PTA) or stenting are performed [7]. These treatments have been shown to effectively recanalize the artery with lower morbidity and mortality than open surgery [8, 9]. Although these techniques demonstrated to be safe and efficient [8–16], the long-term patency of HA, as well as the delayed consequences on the biliary tree, is still a subject of debate [2, 5, 17–21]. Furthermore, because not all patients with HA thrombosis or stenosis develop IC, the identification of protective or predisposing factors is also important.

The aim of this study was to evaluate the long-term outcome of endovascular treatment based on arterial patency by DUS, as well as to evaluate bile duct damage on MRCP.

Materials and Methods

The institutional review board approved this study and informed consent was waived. All patients who underwent endovascular treatment for HA complication after LT between January 2004 and December 2014 were extracted from the picture archiving and communication system (PACS) of our hospital. Clinical, surgical, imaging, laboratory and follow-up data were retrospectively evaluated. Surgical procedures used standard techniques for orthotopic LT or split grafts. Patients presenting with an arterial complication during surgery were immediately treated surgically and were therefore not included in this study.

Diagnosis of HA Complications

In our institution, the diagnosis of HA thrombosis and stenosis was based on a two-step algorithm. Systematic postoperative DUS of the liver graft vasculature was performed daily for the first 5 days, then weekly for 1 month and every 6 months thereafter. Arterial complications were suggested on DUS in the presence of one or more of the following features: (1) post-anastomotic HA resistive index < 0.5; (2) time to peak > 0.08 s); (3) tardus-parvus waveform distal to the stenosis; (4) increased peak systolic velocity (> 200 cm/s) at the stenosis [22] (Fig. 1A). If a complication was suspected, patients rapidly underwent MDCTA (Fig. 1B).

During the study period, MDCT examinations were performed with a bolus tracking technique in the arterial phase and with a 70 s delay in the portal phase with the following parameters: intravenous administration of a 2 mL/kg of non-ionic contrast material (350 mg iodine); injection rate, 4 mL/s with a mechanical power injector; reconstruction slice thickness, 1.25 mm. If the diagnosis of HA thrombosis or stenosis was confirmed, patients were referred to the department of interventional radiology for treatment (Fig. 1C).

Endovascular Techniques

Endovascular treatment included percutaneous transluminal angioplasty (PTA) with or without bailout stenting performed under local anesthesia. In the presence of existing thrombosis of the HA or if thrombosis was discovered during treatment, catheter-directed thrombolysis (CDT) was performed. We favored femoral access and 6-French introducer sheaths to guide catheters. We mainly used low-profile balloons, short self-expanding nitinol stents and balloon-expandable metal stents (Fig. 1C–E). Balloon and stent size ranged from 4 to 5 mm in diameter and 12–20 mm in length. Stenting was only performed in

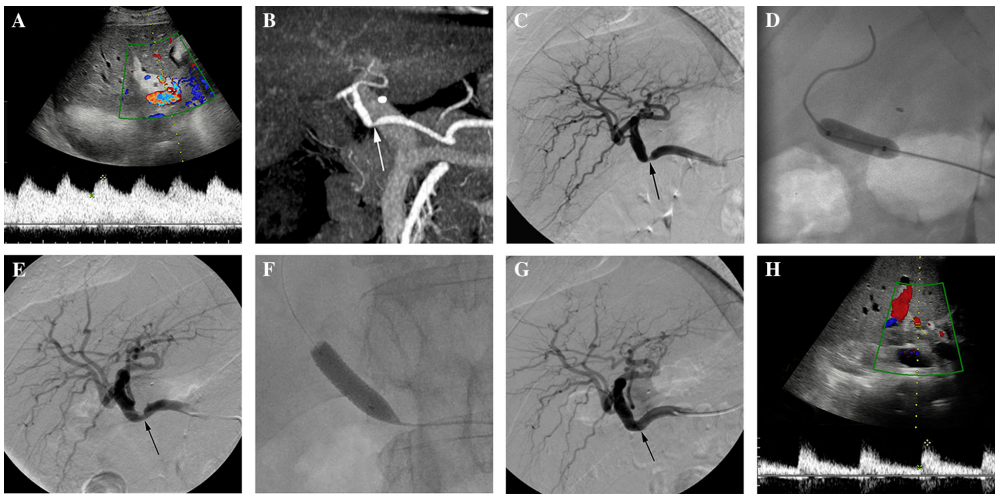

Fig. 1 Endovascular treatment of an anastomotic stenosis of the hepatic artery in a 62-year-old transplanted patient. **A** Doppler-US of the donor hepatic artery showing a tardus-parvus waveform (resistive index, 0.39; time to peak, 0.22 s). **B** Coronal MIP CT image showing a short anastomotic stenosis (arrow). **C** Hepatic angiogram confirming a short stenosis > 70%. **D** Angioplasty with a 5-mm balloon. **E** Control angiogram showing a residual stenosis > 50%. **F** Placement of a balloon-expandable stent (diameter, 5 mm; length, 15 mm). **G** Control angiogram showing absence of residual stenosis. **H** Control Doppler-US showing a normal waveform (resistive index, 0.64; time to peak, 0.06 s)

the presence of residual stenosis or a flow-limiting dissection, and covered stent was used in case of an associated pseudoaneurysm [8, 15] (Fig. 1F, G). CDT was performed with alteplase (Actilyse; Boehringer Ingelheim), in case of acute arterial thrombosis. A bolus of 8 mg was injected manually in the main thrombus via a 4-F multisided hole-dedicated catheter (Fountain infusion system; Merit Medical Systems), followed by a slow infusion of 1 mg/h for 12 h. A second angiogram was performed at the end of the infusion to confirm recanalization. All patients were given anticoagulation therapy during the procedure including an intra-arterial bolus dose of 45 IU/kg of unfractionated heparin. After the procedure, patients received 100 mg of acid acetylsalicylic per day for life. Clopidogrel 75 mg/day was added for three months if a stent was placed.

Follow-Up Surveillance

Patients were followed up until death, lost to follow-up or last follow-up. The study ended on October 2016. HA patency was assessed by DUS every 6 months with the same criteria as for postoperative DUS (Fig. 1H). Early and late restenosis were defined as recurrent HA stenosis less or more than 1 month after angioplasty, respectively [10]. MR imaging at 1.5-T, including MRCP, was performed either yearly for LT follow-up, or for investigation of biliary complications suspected on clinical and biological findings. The protocol for MRCP included radial 2D single shot breath-hold and 3D turbo spin echo T2-weighted sequences. IC was defined as the development of biliary anomalies, excluding biliary anastomotic strictures and recurrent sclerosing cholangitis, such as (1) extra- or intrahepatic ductal strictures; (2) extra- or intrahepatic ductal dilatations; (3) intrahepatic biloma; (4) complete biliary destruction, with irregular dilatation of the entire biliary tree and presence of biliary casts [23–25] (Fig. 2A–F). To confirm that patients were properly screened and reduce a possible observation bias, all survivors were recalled to undergo both DUS and MRCP, the former to assess HA patency, and the latter to evaluate the presence of biliary anomalies. When patients died or were lost to follow-up before the end of the study, we analyzed the last DUS and MRCP retrieved from the PACS. A consensus analysis of images was obtained from two abdominal radiologists with 10 and 15 years of experience (blinded).

Data Analysis

Results are presented as mean ± standard deviation or median and range for quantitative data, and as the number of cases (percentage of cases) for categorical variables. Comparisons were performed with the Student-*t* test and

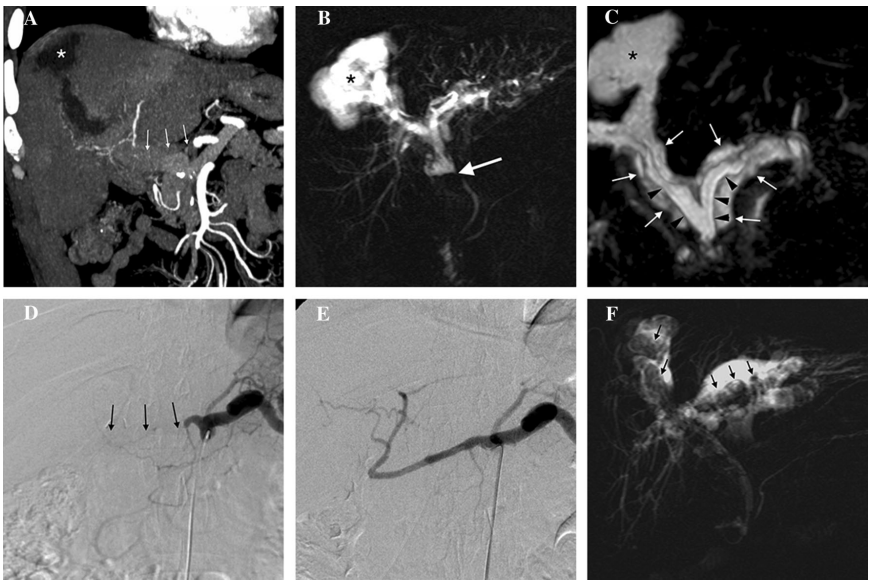

Fig. 2 Ischemic cholangiopathy following occlusion of the hepatic artery 8 months after liver transplantation in a 52-year-old patient. **A** Coronal MIP CT image showing an occlusion of the hepatic artery (arrows) and an intrahepatic biloma (asterisk). **B** MR cholangiogram showing an irregular dilatation of the donor biliary tract, extending to the anastomosis and sparing recipient biliary main duct (arrow). **C** 3D-MRCP image showing the abnormal bile ducts (black arrowheads), surrounded by periportal fluid (white arrows) communicating with the biloma (asterisk). **D** Hepatic angiogram confirming a complete occlusion of the hepatic artery (arrows). **E** Control angiogram after catheter-directed thrombolysis and placement of a nitinol stent (diameter, 4 mm; length, 20 mm) showing patency of the hepatic artery. **F** Follow-up MR cholangiogram 3 weeks after endovascular treatment showing progression of the biliary destruction with casts formation (arrows)

the Mann–Whitney *U* test for continuous variables. Qualitative data were compared with the Chi-square or Fisher's exact tests when necessary. Patient characteristics including demographic, surgical, interventional, clinical and laboratory data associated with the occurrence of IC were identified. Survival time corresponded to the delay between the date of the endovascular procedure and death, the end of the study period or the last visit. Survival curves were calculated using the Kaplan–Meier method and compared using the log-rank test. Variables were analyzed by the receiver-operating characteristic (ROC) to determine the optimal cut-off time for post-transplant arterial complications, and Kaplan–Meier curves were reconstructed using this cut-off value. Tests were always two-sided, and $p < 0.05$ was considered significant. All analyses were performed using Statistical Package for the Social Sciences (SPSS) software (version 20.0, IBM SPSS Inc).

Results

Patients' Characteristics

Fifty-three consecutive patients (mean age, 50 ± 12 years; 42 males) were included in this study and represented 5.05% of the transplanted population ($N = 1048$) in our institution during the study period. Forty-one (77%) transplantations were orthotopic LT, and 12 (23%) were split grafts. Forty-seven (88%) patients had bilio-biliary anastomosis and 6 (12%) had bilio-enteric anastomoses. Most arterial anastomoses were end-to-end between the HA of the recipient and the donor ($n = 40$, 83%). At transplantation, the MELD score was 14.0 ± 7.6, the age of liver donor was 55 ± 17 years, and the cold/warm ischemic time was 502 ± 158 min, and 50 ± 15 min, respectively.

HA Complications and Endovascular Treatments

The indications for primary endovascular treatment are described in Table 1. The initial endovascular procedures in the 53 patients were PTA in 18 (34%), PTA with bailout stenting in 32 (60%) and PTA with or without stenting and CDT in 3 (6%). The primary technical success rate was 94% (n = 50). The technical failures were impossibility to cross a non-anastomotic stenosis (n = 1), arterial tortuosity (n = 1), and median arcuate ligament with associated stenosis (n = 1). Treatment was attempted again in all three patients with one secondary success (arterial tortuosity) and two failures. Six (11%) adverse events were recorded, including one post-procedural major complication (a pseudoaneurysm detected and treated by a covered stent the following day), and five peri-procedural minor complications (four acute HA thromboses immediately treated by CDT and one HA dissection treated by stenting).

Forty-two (79%) patients underwent one endovascular procedure, nine had two procedures (17%), one had three procedures (2%) and one had four procedures (2%), for a total of 14 re-interventions. Most re-interventions were performed for restenosis of the HA (N = 10). The patency rate of HA at 1-month, 6-month, 1-year, 3-year and 5-year, was 89, 87, 83, 81 and 81%, respectively.

Table 1 Endovascular procedures

First endovascular intervention (n= 53)	
Indications for first intervention	
Hepatic artery stenosis	49 (92%)
Anastomotic	45 (85%)
Non-anastomotic	4 (8%)
Hepatic artery kinking with associated stenosis	3 (6%)
Hepatic artery thrombosis	1 (2%)
Primary technical success rate	47 (94%)
Secondary technical success rate	48 (96%)
Major complications rate	1 (2%)
Minor complications rate	5 (9%)
Re-intervention (n = 14)	
Indications for re-intervention	
Early* restenosis	2 (14%)
Late* restenosis	8 (57%)
Early* thrombosis	1 (7%)
Late* thrombosis	1 (7%)
Persistent kinking	1 (7%)
Pseudoaneurysm	1 (7%)
Primary technical success rate	13 (93%)
Complications rate	0 (0%)

*Early/late restenosis/thrombosis </> 1 month

Follow-Up and Factors Associated with Ischemic Cholangiopathy

The mean follow-up for the entire cohort was 58 months (16–124). The median delay between surgery and the diagnosis of arterial complications was 5.4 months (0.1–63.6), and the median delay between diagnosis and endovascular treatment was 13 days (1–90). Thirty-six patients (68%) were recalled for DUS and MRCP. Thirteen (25%) of the remaining patients died, two were lost to follow-up and two refused to undergo MRCP. The causes of death were recurrent hepatocellular carcinoma (n = 6), postoperative complications after re-transplantation (n = 2), severe acute pancreatitis (N = 1), IC (n = 1), multiple organ failure (n = 1), lymphoma (n = 1) and chronic rejection (n = 1).

DUS examinations were normal according to the defined criteria in 38 (72%) patients and abnormal in the remaining 15 (28%) patients. Features of IC were found in 17 (32%) patients at MRCP, and the remaining 36 (68%) patients had no biliary abnormalities. The two patients who did not undergo MRCP had no symptoms, normal liver tests and absence of biliary abnormalities at MDCT. Ten of the 36 (26%) patients with normal DUS on follow-up developed signs of IC, and 28 (74%) did not, whereas seven of the 15 patients (47%) with abnormal follow-up DUS developed features of IC, and 8 (53%) did not (p = 0.197). Patients with IC had a shorter median survival than those without (77 months (95% CI 53–101) versus 133 months (95% CI 117–148), p = 0.0163) (Fig. 3). A cut-off value of 3 months between LT and arterial complications identified two groups of patients, with a significantly different risk of developing IC (42% [n = 15] vs. 12% [n = 2], for

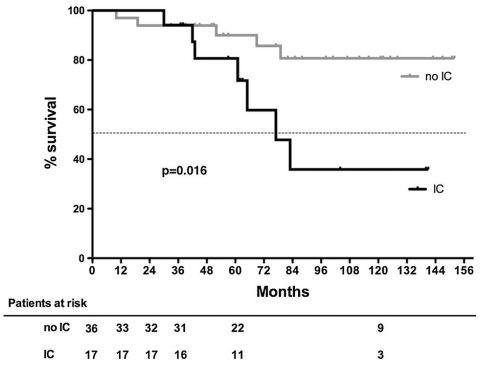

Fig. 3 Survival curves (Kaplan–Meier method) according to the occurrence of ischemic cholangiopathy. *IC* ischemic cholangiopathy

Table 2 Comparison of patients, surgical and endovascular characteristics according to the occurrence of ischemic cholangiopathy

Characteristics	No IC $n = 36$	IC $n = 17$	p value
Patients			
Male ($n = 42$)	30 (71%)	12 (29%)	0.301
Female ($n = 11$)	6 (55%)	5 (45%)	
Age (mean ± SD)	50 ± 12	49 ± 12	0.717
*Underlying disease**			
Alcohol ($n = 15$)	12 (80%)	3 (20%)	0.633
NAFLD ($n = 3$)	3 (100%)	0 (0%)	
HCC ($n = 22$)	16 (73%)	6 (27%)	
HBV ($n = 7$)	4 (57%)	3 (43%)	
HCV ($n = 19$)	12 (63%)	7 (13%)	
Other ($n = 16$)	10 (63%)	6 (37%)	
Surgical technique			
Total liver ($n = 41$)	28 (68%)	13 (32%)	0.916
Split liver ($n = 12$)	8 (67%)	4 (33%)	
Bilio-biliary anastomosis ($n = 47$)	33 (70%)	14 (30%)	0.318
Bilio-enteric anastomosis ($n = 6$)	3 (50%)	3 (50%)	
Standard arterial anastomosis ($n = 40$)**	26 (65%)	14 (35%)	0.892
Complex arterial anastomosis ($n = 8$)**	5 (63%)	3 (37%)	
Timing LT-diagnosis			
< 3 months ($n = 36$)	21 (58%)	15 (42%)	0.028
> 3 months ($n = 17$)	15 (88%)	2 (12%)	
Initial intra-arterial intervention ($n = 53$)			
PTA ($n = 18$)	13 (72%)	5 (28%)	0.802
PTA + stent ($n = 32$)	21 (68%)	11 (34%)	
PTA + CDT ($n = 1$)	1 (100%)	0 (–)	
PTA + stent + CDT ($n = 2$)	1 (50%)	1 (50%)	
Initial technical success			
Yes ($n = 50$)	33 (66%)	17 (33%)	0.278
No ($n = 3$)	3 (100%)	0 (0%)	
Arterial complications			
Yes ($n = 6$)	3 (50%)	3 (50%)	0.520
No ($n = 47$)	33 (70%)	14 (30%)	
Follow-up DUS			
Normal ($n = 36$)	26 (72%)	10 (28%)	0.197
Abnormal ($n = 14$)	7 (50%)	7 (50%)	

IC ischemic cholangiopathy, *NAFLD* non-alcoholic fatty liver disease, *HCC* hepatocellular carcinoma, *HB/CV* hepatitis B/C virus, *LT* liver transplantation; (d/y) days/years, *PTA* percutaneous transluminal angioplasty, *CDT* catheter-directed thrombolysis, *DUS* duplex ultrasound

*Diseases can be concomitant

**Data available for 48 patients

complications < 3 and > 3 months, respectively, $p = 0.028$) (Table 2). No other factors were shown to be associated with the development of IC.

Discussion

This study confirmed the feasibility and safety, as well as the good long-term outcome of endovascular treatment for HA complications secondary to LT, with 72% of patients showing normal DUS results of the HA. These results are similar to other studies showing patency rates of between

77 and 79% [9, 10, 12, 13, 22]. Nevertheless, around one-third of our patients developed IC, which is also consistent with previous reports [2, 20, 21, 26]. The only factor associated with the occurrence of IC identified in this study was the delay between LT and the onset of arterial complications. These results suggest that patients with arterial complications related to LT, especially within 3 months after surgery, had a greater risk of developing IC. Interestingly, no other factors were found to be associated with the development of IC. Technical factors, including initial technical success, peri- and post-procedural complications or the recurrence of arterial anomalies were not associated with a higher risk of developing IC. If the arterial complication occurred within the first three months after transplantation, the risk of IC is 42%, whatever the presence or not of Doppler abnormalities.

In 2015, Pulitano et al. [21] published a study comparing biliary complication-free survival of patients treated, or not, for HA stenosis after LT. The authors showed that endovascular treatment of the HA was beneficial if the stenosis developed within 6 months after LT. They also estimated that patients were unlikely to develop biliary complications after this critical period and thus would not benefit from recanalization [21]. The present results partially support these findings and thus sustain the hypothesis that patients may develop arterial collateral vessels over time. Interestingly, long-term follow-up showed that one-third of patients who developed IC had normal DUS results of HA, and half of the patients with abnormal DUS did not develop IC. This suggests that causes aside from impaired arterial inflow to the peribiliary plexus itself [19] must also be present to develop IC.

Nevertheless, despite prompt endovascular treatment, transplanted patients with vascular complications still develop biliary ischemic lesions more frequently than those without (3–17% in published studies versus 32% in the present study) [17]. Nevertheless, our rate of biliary complications is comparable to that found in similar series (31.4%) [21] and lower than in patients with arterial complications receiving no treatment (67%) [20]. This also supports the necessity of associated causes for the development of IC, such as donor age > 50 years, greater donor body weight, cytomegalovirus co-infection, recurrent sclerosing cholangitis, ABO-incompatible transplantations, graft rejection, prolonged warm ischemic time, and delayed re-arterialization in case of sequential revascularization during LT [2, 3, 22].

It is important to note that even though the survival rate in patients with IC was lower than that in patients who did not develop biliary lesions in our series, the mortality rate of the entire cohort was similar to that in studies of transplanted patients without IC [27, 28]. This is mainly due to the severity of biliary lesions. Indeed, only a few patients had severe biliary disease that threatened graft survival and most had moderate biliary disease that could be treated percutaneously or endoscopically. This may have been due to the beneficial effect of arterial recanalization on the severity of cholangiopathy, although this is beyond the scope of this study.

This study has several limitations. Besides its retrospective design, the number of included patients was small, as well as the sample size of the different subgroups, decreasing the statistical power of analysis. Nevertheless, this is the largest published cohort evaluating endovascular treatment for consecutive HA complications secondary to LT in well-characterized patients. Also, the absence of a control group and the absence of systematic confirmation of DUS abnormalities by MDCT limits the interpretation of the survival curves. Finally, the accuracy of DUS examinations is variable and dependent on the operator and the correction angle, especially with tortuous HA, yielding false positive results.

In conclusion, systematic endovascular management of arterial impairment after LT is feasible and safe. However, approximately one-third of patients develop biliary ischemic lesions. Even though the mechanism of IC is not clearly understood, our results show that patients who develop post-transplantation arterial complications within 3 months after surgery are at greater risk of developing ischemic lesions, and this subgroup would benefit the most from treatment. Finally, normal DUS of the HA during follow-up does not exclude the presence of IC.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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