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Endoscopic ultrasound in chronic liver disease

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Abstract

Endoscopic ultrasound (EUS) is a minimally invasive diagnostic and therapeutic modality with a number of established as well as evolving uses in patients with chronic liver disease. Compared to other diagnostic tools such as cross-sectional imaging or conventional endoscopy, EUS has been shown to increase diagnostic sensitivity and therapeutic success for many clinical scenarios and applications with a low rate of adverse events. In this review, we discuss and focus on the current and growing role of EUS in the evaluation and/or treatment of hepatobiliary masses, hepatic parenchymal disease, portal hypertension, esophageal and other varices, and indeterminate biliary strictures.

Key words: Endoscopy; Cirrhosis; Liver mass; Liver biopsy; Variceal bleeding

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Core tip: Endoscopic ultrasound (EUS) is a minimally invasive diagnostic and therapeutic modality with numerous existing and emerging applications in patients with chronic liver disease. In this review, we discuss the role of EUS in the evaluation of hepatobiliary masses, hepatic parenchymal disease, portal hypertension, and indeterminate biliary strictures. We also review how EUS can serve as an ancillary tool to conventional endoscopic and other therapies, including the use of EUS for the treatment of variceal bleeding.

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INTRODUCTION

Endoscopic ultrasound (EUS) has been established as a valuable diagnostic tool in gastroenterology since its inception in the 1980s. EUS has proven valuable in patients with liver disease when conventional endoscopy or cross-sectional imaging are insufficient or inconclusive or when surgical interventions pose excessively high risk. In more recent years, EUS has seen an expansion in its therapeutic applications, many of which are germane to the management of chronic liver disease. In this review, we discuss the indications for, performance, impact, and safety of EUS, both diagnostic as well as therapeutic, in patients with chronic liver disease, with a focus on hepatobiliary masses, hepatic parenchymal disease, portal hypertension, esophageal and other varices, and indeterminate biliary strictures.

EUS IN THE EVALUATION OF LIVER MASSES

The differential diagnosis of a liver lesion is broad, with many benign as well as malignant potential etiologies. While the majority of solitary lesions are benign (*e.g.*, hepatic cysts, focal nodular hyperplasia, hepatic adenoma, hemangioma, regenerative nodules), malignant etiologies [*e.g.*, hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), and other metastatic masses] have serious consequences and rely on timely diagnosis^[1]. Accurate characterization and diagnosis of liver masses comprises an important topic and area of research in modern practice, as clinical mimics may exist, and some masses may be particularly challenging to definitively diagnose.

Evaluation of small lesions

Cross-sectional imaging with computed tomography (CT), magnetic resonance imaging (MRI), and transabdominal ultrasound followed by transcutaneous image-guided biopsy is generally the accepted method of evaluation for liver masses^[2]. However, cross-sectional imaging has proven to be less sensitive for smaller (< 10 mm) lesions^[3,4]. For these smaller masses, EUS has been found to have improved sensitivity, with the ability to position the probe closer to the liver surface. A prospective study of 574 patients with gastrointestinal or pulmonary malignancy who underwent EUS found that EUS discovered liver lesions in 14 patients with a mean size of 1.8 cm (range 0.5 cm to 5.8 cm), while CT was only able to identify 3 of the lesions^[5]. Further studies have supported the observation that EUS can identify liver lesions smaller than 5 mm in diameter, many of which may be missed by CT^[6,7]. EUS has also been shown to detect more metastatic lesions compared to CT and is capable of characterizing lesions that are too small to characterize by CT^[8] (Figure 1). Indeed, in a retrospective study of 336 patients who underwent EUS for a malignant diagnosis, EUS was able to detect smaller liver metastases compared to CT scan (mean 8.8 mm *vs* 15.3 mm, respectively)^[9]. There are little data regarding the comparison of EUS and MRI for the detection of small lesions; however, MRI is generally considered more sensitive than CT, and in one study, appeared to have similar diagnostic accuracy as EUS^[10].

Performance of EUS in the evaluation of liver masses

The sensitivity of EUS has been examined and validated by multiple studies. DeWitt *et al*^[11] reviewed 77 malignant and benign liver lesions that underwent EUS-guided fine needle aspiration (FNA) using a 22-gauge needle (mean 3.4 passes) and found the sensitivity of EUS-FNA to be between 82% and 94%. In a prospective study of 41 patients with known or suspected malignancy and concomitant liver lesions, EUS-FNA was successfully performed in 40 of 41 patient using a 22-gauge needle and a mean of 1.4 passes (in one patient, the authors report it was not possible to aspirate sufficient material)^[12]. For malignant lesions, a combination of cytology and histology yielded a sensitivity and specificity of 94% and 100%, respectively^[12].

Recently, EUS criteria have been proposed to select liver lesions that may be

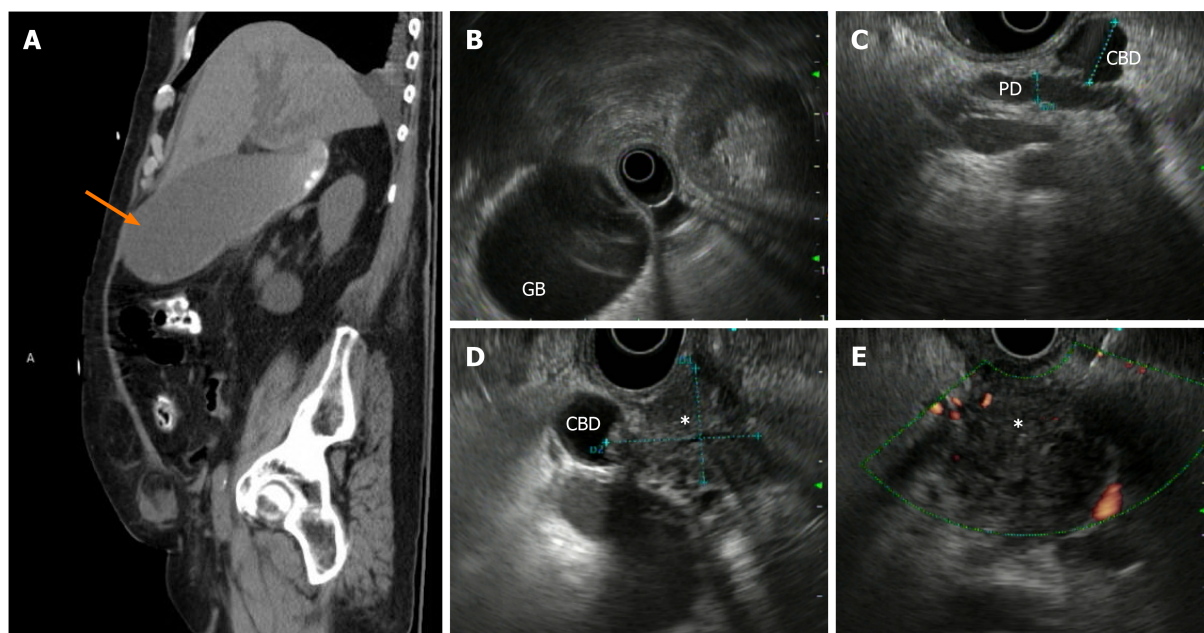


Figure 1 Endoscopic ultrasound in the diagnosis of obstructive jaundice. An 80-year-old male with a history of non-alcoholic fatty liver disease presented with new onset of painless jaundice, physical examination consistent with Courvoisier's sign (palpable gallbladder), and laboratory test results suggestive of severe biliary obstruction. A: Distended gallbladder (arrow) seen on computed tomography, sagittal view. B: Distended gallbladder seen on endoscopic ultrasound. C: Double duct sign consisting of a dilated common bile duct (CBD) and dilated pancreatic duct. D: A poorly-margined, hypoechoic pancreatic mass (asterisk) invading the distal CBD. E: Fine-needle biopsy of the pancreatic mass (asterisk), which led to tissue diagnosis of adenocarcinoma and facilitated subsequent management. GB: Gallbladder; PD: Pancreatic duct; CBD: Common bile duct.

malignant and need to be sampled. Derived from a retrospective review of a cohort of 100 patients, features suggestive of benign masses were hyperechogenicity and distinct geographic shape (Figure 2) while those suspicious for malignancy included masses with two components, presence of post-acoustic enhancement, distortion of adjacent structures, hypoechogenicity, and size ≥ 10 mm^[9]. These criteria were subsequently validated in a separate cohort of 100 patients with pathology or imaging as the gold standard and then used to generate a 16-point scoring system based on tested criteria. Using a cut-off of 3 points, the combined sensitivity, specificity, and positive predictive value (PPV) in predicting a malignant hepatic mass was found to be 85%, 82%, and 88%, respectively^[9].

In addition to being an effective diagnostic tool, especially for smaller liver lesions, EUS-guided fine needle biopsy (FNB) also appears to be an effective “rescue” method when percutaneous tissue acquisition has failed or been deemed unsafe. A study of 23 patients who needed a pathological diagnosis of a liver mass who failed percutaneous biopsy or where percutaneous biopsy was contraindicated (due to coagulopathy, ascites, inadequate sampling, or lack of visualization by cross-sectional imaging) found that EUS-FNB with a 22-gauge core biopsy needle (except for one patient in whom a 25-gauge needle was used) was a reliable alternative^[13]. EUS-FNB was technically successful in 21 of the 23 lesions (93%), adequate tissue for pathology was obtained in 19 patients, and the overall diagnostic accuracy for malignancy and specific tumor type were 90.5% and 85.7%, respectively, with a median of 2 passes (range 1 to 5) during biopsy. None of the patients had adverse events related to the procedure^[13].

Though CCA may also present as a liver mass, the role of EUS in the management of CCA is less clear. A 2014 systemic review and meta-analysis identified six studies (196 patients) that investigated the role of EUS for the detection of CCA where biopsy was available as the gold standard^[14]. The overall pooled sensitivity in 196 patients was 66%. In five of the six studies, EUS identified a mass in 25% to 100% of patients; one study did not report data regarding the presence of a mass. The pooled sensitivity of EUS for CCA in studies that detected a mass on EUS (146 patients) was 80%^[14].

EUS-GUIDED LIVER BIOPSY FOR THE EVALUATION OF LIVER PARENCHYMA

Despite advances in the biochemical and imaging-based evaluation of parenchymal

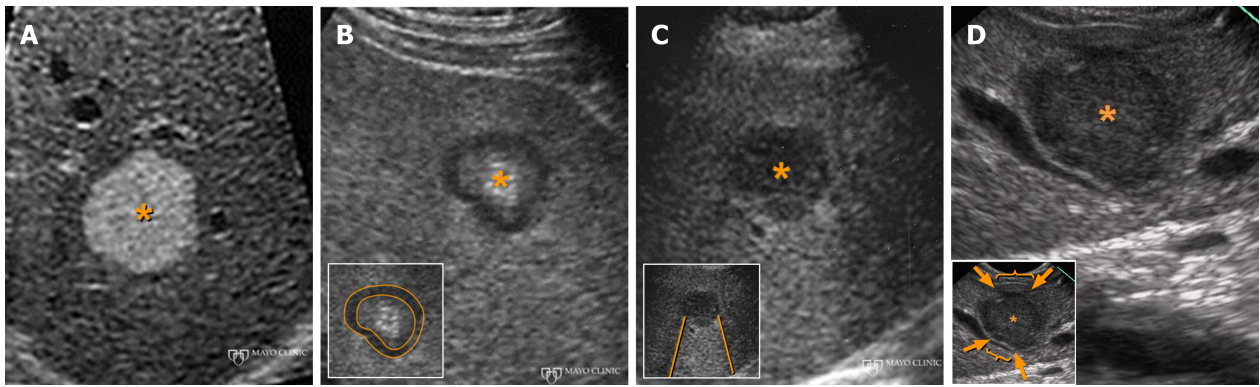


Figure 2 Characteristics of benign and malignant liver masses. A: A distinctly demarcated hyperechoic lesion consistent with a benign hemangioma. B: A liver lesion with both iso/hypoechoic parts peripherally (outlined in orange in inset) and central hyperechoic parts suggestive of malignancy. C: A hypoechoic mass exhibiting post-acoustic enhancement (outlined in orange in inset) as frequently seen in malignancy. D: A hypoechoic, poorly demarcated mass distorting adjacent strictures (orange arrows and brackets in inset) suggestive of malignancy.

liver disease, a liver biopsy is still frequently needed to determine the etiology and grade the severity of liver pathology. Microscopic examination of hepatic tissue is often a requisite step in the workup after other tests, including serology, imaging, and endoscopy, have failed to provide a diagnosis. Traditionally, a percutaneous or transjugular approach has been used to obtain a liver biopsy^[15,16]. In addition to sonographic and other hepatic imaging data that can be obtained *via* EUS, in recent years, EUS-guided liver biopsy (Figure 3) has become an alternative to traditional methods of liver biopsy as it allows for examination of the upper gastrointestinal tract, pancreas, and the biliary tree with ultrasonic visualization of the liver, while also allowing for acquisition of tissue during the same session. This modality thus allows for a one step diagnosis in patients being referred for abnormal serum liver tests who also have an indication for upper endoscopy.

Performance of EUS-guided parenchymal liver biopsy

In the earliest example of EUS-guided liver sampling, 2 patients underwent EUS-guided biopsy of the liver using a Tru-Cut biopsy (TCB) needle (Cook Medical, Bloomington, IN, United States) as part of the evaluation for abnormal liver tests^[17]. Subsequently, a retrospective study found that TCB was able to provide adequate tissue for histological diagnosis in 100% of patients^[18]. However, the high success rate was not able to be reproduced in a prospective case series where adequate tissue was obtained in only 19% of patients^[19]. This low success rate was thought partly to be due to the small size and stiffness of Tru-Cut needles used.

In 2012, a prospective case series of 22 patients undergoing same-session EUS and liver biopsy using a 19-gauge FNA needle [EchoTip® (Cook Medical, Bloomington, IN, United States)] was able to obtain adequate tissue in 91% (20/22) of patients (with mean portal tract count of 9 and aggregate specimen length of 36.9 mm), demonstrating that EUS-guided liver biopsy could be successfully performed with a regular 19-gauge FNA needle^[20]. A large multicenter trial of 110 patients confirmed efficacy and feasibility of using a 19-gauge needle [Expect™ or Expect™ Flexible (Boston Scientific, Marlborough, MA, United States)]^[21]. Median length of specimens was 38 mm (0 mm - 203 mm), and 105 patients had specimens with over six complete portal triads (PTs) and length > 15 mm. Pathological diagnosis was possible in 108/110 (98%) of cases. One patient developed a subcapsular hematoma but did not require further intervention to control bleeding. This study was limited by the fact that only five patients were found to have cirrhosis which is important as specimen fragmentation has been reported to occur at higher rates in patients with cirrhosis, resulting in decreased specimen adequacy^[22].

Over the years, additional studies have been performed to compare various biopsy needles and refine the EUS-guided liver biopsy technique. A summary of these studies is detailed in Table 1. Studies which have compared needle size have generally found that a 19-gauge needle is superior to smaller 20- or 22-gauge needles due to the significant drop in specimen adequacy rate with smaller needles^[23-25]. In a randomized study comparing a 19-gauge Expect™ Flexible needle (Boston Scientific) versus a 22-gauge SharkCore™ [Medtronic, Minneapolis, MN] needle in 80 patients, the 19-gauge needle produced more adequate specimens than the 22-gauge needle (88% *vs* 27%, respectively), primarily attributed to greater tissue fragmentation with the 22-gauge needle^[23]. Use of a heparin-primed needle has also been reported to

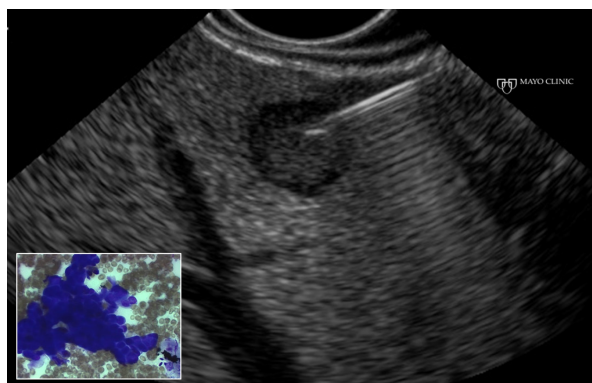


Figure 3 Endoscopic ultrasound-guided fine needle biopsy of a hypoechoic hepatic lesion first seen on non-invasive imaging; cytopathology was consistent with metastasis from pancreatic ductal adenocarcinoma (inset).

improve tissue adequacy compared with dry needle techniques^[26]. In a prospective crossover study evaluating various suction techniques in 120 biopsy specimens from 40 participants, specimen adequacy rate was 98%, 93%, and 80% in the wet heparin (needle flushed with heparin), dry heparin (needle flushed with heparin then flushed with air), and dry needle (no heparin used) groups. The use of heparin has been shown to be safe and not interfere with specimen processing^[27], and the improved yield is thought to be due to the reduction in blood clot formation within the biopsy needle with the use of heparin^[26].

Safety of EUS-guided liver biopsy

To date, there are no head-to-head comparisons of liver lesion biopsy performed under the guidance of cross-sectional imaging versus EUS in a randomized control study. However, a recent retrospective study of 30 patients who underwent EUS-guided liver biopsy and 60 patients who underwent percutaneous liver biopsy found that EUS-guided liver biopsy was associated with a significantly shorter hospital stay (median time of hospital stay 3 h *vs* 4.2 h) and less pain (median pain score 0 *vs* 3.5)^[28]. In this study, no patients had significant adverse events. The risk of adverse events with EUS-FNA appears to be comparable to the adverse event rate of FNAs of other types of lesions. In a systematic review of 51 studies (10941 patients), the overall rate of adverse events in patients undergoing EUS-FNA of liver lesions was 2.3% (8/344), compared to 3.6% and 2.8% for ascites and pancreatic cystic lesions, respectively^[29]. A more recent retrospective study reported a similar adverse event rate of 2.9%^[9]. In contrast, a retrospective study of 3357 percutaneous liver biopsies over 36 years reported an adverse event rate of 4%^[30]. Adverse events after EUS-FNA of liver lesions include abdominal pain, nausea, fever, bleeding, duodenal perforation, and death as summarized in Table 2.

EUS ELASTOGRAPHY

Elastography generally refers to an imaging modality that assesses for changes in the elasticity of tissue, as can be seen with fibrotic, inflammatory, or malignant processes. Reduced elastic rebound suggests stiffer tissue, which in the context of liver disease, tends to be an indicator of fibrosis, cirrhosis, or other pathologic processes. Elastography has been shown to have a high correlation with the degree of histologic fibrosis and can also be helpful in the assessment of sequelae of advanced fibrosis and cirrhosis such as the presence of varices, risk of variceal rupture, and prediction of HCC development^[31].

Traditionally, transabdominal ultrasound has been the platform for hepatic elastography technique. However, transabdominal elastography is often limited by ascites, body habitus, and narrow intercostal spaces^[31]. EUS elastography (EUS-EG) can overcome many of these aforementioned limitations. Although originally developed to examine deeper abdominal tissues (*e.g.*, pancreas), recent studies have found that it can also be useful in the assessment of chronic liver disease, and in particular, solid liver masses^[32]. In a recent prospective study of 50 patients, Schulman *et al*^[33] found that EUS-EG was able to distinguish between normal, fatty, and cirrhotic tissue with a strong predictive value (area under the receiver operating characteristic curve, 0.865). In this study, the use of EUS-EG added a mean of 5 mins to the

Table 1 Comparison of needle performance in endoscopic ultrasound-guided liver biopsies

Ref.	Design	Exclusion criteria	Needle	Median (range) number of complete portal tracts	Median (range) aggregate specimen length, mm	Median (range) number of passes	Adequacy (%)	Complications (number of patients)
Human studies								
Stavropoulos <i>et al</i> ^[20] , 2012	Prospective (<i>n</i> = 22 patients)	Suspected/known malignancy; Platelet < 50, INR > 1.5; Use of antiplatelets within 7 days; Inability to provide informed consent; Pregnancy	19-G EchoTip® FNA (Cook Medical)	9 (1-73)	36.9 (2-184.6)	2 (1-3)	91%	None
Diehl <i>et al</i> ^[21] , 2015	Prospective, non-randomized (<i>n</i> = 110 patients)	Malignant liver disease; Platelet < 50, INR > 1.5; Use of antiplatelets within 5 days; Inability to provide consent; Pregnancy	19-G Expect™ FNA (Boston Scientific) or 19-G Expect™ Flexible FNA (Boston Scientific)	14 (0-68)	38 (0-203)	1 or 2	98%	Pericapsular hematoma (1)
Sey <i>et al</i> ^[82] , 2015	Cross-sectional (<i>n</i> = 75 patients)	Liver lesion or presence of varices Prior upper GI or liver surgery; Use of antiplatelets not held prior to procedure; Platelet < 50, INR > 1.5	19-G EchoTip® ProCore FNB (Cook Medical) (<i>n</i> = 30)	5 (0-24)	20 (5-60)	2 (1-3)	97%	None
			19-G Quick-Core® FNB (Cook Medical) (<i>n</i> = 45)	2 (0-15)	9 (0-25)	3 (1-7)	73%	Abdominal pain (2)
Shah <i>et al</i> ^[83] , 2017	Retrospective (<i>n</i> = 24 patients)	Not stated	19-G SharkCore™ FNB (Medtronic)	31.5 (5-85)	65.6 (17-167.4)	2	87.5%	Abdominal Pain (2); Subcapsular bleeding (1)
Mok <i>et al</i> ^[23] , 2018	Randomized cross-over (<i>n</i> = 80 patients)	Platelets < 50, INR > 1.5; Diagnosis of cirrhosis ; Under 18 years age; Inability to provide informed consent; Pregnancy	19-G Expect™ Flexible FNA (Boston Scientific)	7.4 ¹³	76.5 ¹³	1	88%	None
			22-G SharkCore™ FNB (Medtronic)	6.19 ¹³	66.91,3	1	68%	Abdominal Pain (1)
Cadaveric studies								
Lee <i>et al</i> ^[84] , 2017	Nonrandomized (<i>n</i> = 2 livers)	N/A	19-G EchoTip® ProCore FNB (Cook Medical)	3.33 ¹	473 ²			N/A
			19-G EZ Shot 2 FNA (Olympus Corporation)	4.00 ¹	298 ²			
			19-G Expect™ Slimline FNA (Boston Scientific)	4.42 ¹	426 ²			
			19-G SharkCore™ FNB (Medtronic)	8.83 ¹	507 ²			

Schulman <i>et al</i> ^[25] , 2017	Randomized (<i>n</i> = 2 livers)	N/A	18-G TruCore™ (Argon Medical Devices)	7.00 ¹	155 ²		
			19-G SharkCore™ FNB (Medtronic)	6.2 ¹		85.4%	N/A
			22-G SharkCore™ FNB (Medtronic)	3.8 ¹		85.4%	
			19-G EchoTip® ProCore FNB (Cook Medical)	1.7 ¹		About 19%	
			19-G Expect™ FNA (Boston Scientific)	1.9 ¹		About 46%	
			18-G Quick-Core® FNB (Cook Medical)	2.5 ¹		83.3%	
			18-G Coaxial Temno® (CareFusion)	3.5 ¹		81.3%	
			Bovine studies				
Eskandari <i>et al</i> ^[24] , 2019	Nonrandomized (<i>n</i> = 1 bovine liver)	N/A	19-G Acquire™ FNB (Boston Scientific)	11.8 ¹	71.30 ¹	5	N/A
			22-G Acquire™ FNB (Boston Scientific)	6.4 ¹	44.94 ¹	5	
			19-G SharkCore™ FNB (Medtronic)	10.4 ¹	51.50 ¹	5	
			22-G SharkCore™ FNB (Medtronic)	1.4 ¹	20.89 ¹	5	
			19-G EZ Shot 3 Plus FNA (Olympus Corporation)	10.2 ¹	71.77 ¹	5	
			20-G EchoTip® ProCore FNB (Cook Medical)	7.2 ¹	79.79 ¹	5	

¹Mean.²Total specimen length.³Results not statistically significant. INR: International normalized ratio; G: Gauge; FNA: Fine needle aspiration; FNB: Fine needle biopsy; N/A: Not applicable.

procedure, and none of the patients had any periprocedural adverse events; however, this study was limited by the fact that not all patients had corresponding biopsy data. With the ability to evaluate for hepatobiliary masses, parenchymal liver abnormalities, and complications of portal hypertension (*e.g.*, varices), EUS-EG may improve efficiency and reduce the number of procedures when more than one organ requires evaluation. As data on this relatively new modality are limited, additional studies are needed prior to its use in clinical practice.

EUS-GUIDED TREATMENT OF HEPATIC LESIONS

In addition to be a diagnostic modality, EUS has been found to be an effective tool in the treatment of hepatic lesions. The use of EUS may facilitate more targeted interventions (in part as a result of closer proximity between the EUS probe and the lesion of interest) as well as shorter recovery time compared with percutaneous approaches (by eliminating the need to puncture the skin)^[34].

Treatment of cystic liver lesions

Table 2 Most frequent adverse events associated with endoscopic ultrasound-guided liver biopsy

Adverse event	Event frequency/number of patients in the study
Abdominal pain or nausea	7/499 ^[9,85]
Fever	2/167 ^[85]
Bleeding	1/167 ^[85]
Duodenal perforation	2/332 ^[9]
Death	1/167 ^[85]

Simple hepatic cysts are benign lesions that are commonly found incidentally on routine imaging, with most patients asymptomatic and without need for further intervention^[35]. However, larger cystic lesions can cause abdominal pain and distension, among other symptoms or complications, resulting in the need for further management. Surgical therapy has traditionally been regarded as the treatment of choice for symptomatic hepatic cystic lesions, though this intervention carries considerable morbidity^[36,37]. Percutaneous aspiration can be considered in certain cases but is frequently associated with cyst recurrence^[38]. Ethanol lavage therapy (either *via* percutaneous approach or EUS-guided) has recently been found to be an effective and safe alternative to conventional surgical and percutaneous aspiration therapies^[39,40]. While percutaneous ethanol lavage is generally more feasible for right-lobe hepatic cysts, the EUS-guided approach appears to be particularly useful for left-lobe cysts. Furthermore, the EUS-guided approach appears to have better outcomes compared to the percutaneous approach. In a study of 17 patients with hepatic cysts undergoing percutaneous or EUS-guided aspiration and ethanol lavage, patients who underwent EUS-guided sclerotherapy had a higher median reduction in cyst volume (100% *vs* 97.5%, $P = 0.011$), a higher number of completely resolved cysts within 1 year (5 out of 8 patients *vs* 0 out of 10 patients, $P = 0.005$), and a shorter hospital stay (4.5 d *vs* 6.5 d, $P = 0.048$) compared with patients who underwent a percutaneous approach^[39]. EUS-guided drainage (as with percutaneous drainage) also appears to be an effective treatment for infected (known or suspected) hepatic cysts^[41].

EUS-guided drainage of non-hepatic collections (*e.g.*, pancreatic pseudocysts and walled-off necrosis^[42]) are technically essentially the same for patients with and without chronic liver disease and thus is not discussed in the present review.

Treatment of solid liver lesions

Solid hepatic masses include abscesses and malignancies. Similar to the treatment of cystic lesions, solid masses have traditionally been treated with surgical or percutaneous drainage; however, morbidity and mortality is relatively high with these approaches^[43]. In recent years, EUS-guided drainage of liver abscesses has been found to be both safe and feasible, with a lower rate of adverse events and a shorter hospital stay compared with percutaneous drainage^[34]. For hepatic metastases, EUS-guided ablation using ethanol appears to be a viable alternative treatment option to traditional therapies and has been found to result in clinical success in a number of cases^[44-46]. Other experimental treatments utilizing EUS, including EUS-guided neodymium:yttrium-aluminum-garnet laser ablation and EUS-guided fiducial placement for stereotactic body radiation therapy have also been reported as safe and accurate minimally invasive methods of treating hepatic malignancies^[47,48]. However, well-designed prospective studies are needed prior to the use of these novel therapies in clinical practice.

EUS IN PRIMARY SCLEROSING CHOLANGITIS

With advances in MRI technology, magnetic resonance cholangiopancreatography (MRCP) has generally replaced endoscopic retrograde cholangiopancreatography (ERCP) as the initial diagnostic as well as surveillance modality for primary sclerosing cholangitis (PSC)^[49,50]. However, the sensitivity of MRCP is not without limitation, with one systematic review suggesting a sensitivity of MRCP of only 86%^[51]. This has led to efforts to develop a less invasive but more accurate endoscopic modality to diagnose PSC^[52]. In a prospective controlled study, patients with PSC had a larger mean ductal wall thickness compared with patients with uncomplicated inflammatory bowel disease (IBD) or cholelithiasis^[53]. In another study, four EUS criteria (wall thickening ≥ 1.5 mm, irregular wall structure, significant changes of the

caliber of the common bile duct, and perihilar lymphadenopathy) were found to assist with the diagnosis of PSC in 33 patients with cholestatic liver enzyme elevation and either concurrent IBD or positive perinuclear antineutrophil cytoplasmic antibodies^[54]. The authors found a sensitivity and specificity of 76.4% and 100%, respectively for the diagnosis of PSC if two out of the four aforementioned EUS criteria were present.

With regard to complications of PSC, indeterminate strictures pose a hallmark lesion and a frequently challenging entity from a clinical perspective. A recent systemic review and meta-analysis of eight studies including 294 patients found EUS to have superior sensitivity compared with ERCP with brushing and forceps biopsy in the diagnosis of indeterminate biliary strictures (75% *vs* 49%, respectively)^[55]. EUS sensitivity is dependent on the location of the stricture (higher sensitivity for more distal strictures) and the underlying etiology (higher sensitivity for pancreatic cancer compared to CCA, for example)^[56,57]. Thus, it has been proposed that stricture-location should be considered when deciding which diagnostic modality to use; when EUS is used for distal biliary strictures, irrespective of underlying PSC, its accuracy for malignancy detection has been reported to be as high as 96%^[58].

However, despite these studies, EUS is still infrequently used as a diagnostic tool in PSC. There is also uncertainty on whether EUS is practical from a cost perspective. A cost-effective analysis found that an EUS instead of ERCP for indeterminate biliary strictures results in 0.13 additional QALYs (quality adjusted life years), but with an added cost of \$2773.69^[59]. However, after taking into consideration the increased sensitivity of EUS *vs* ERCP (74% and 42%, respectively), the study authors found EUS to be more cost-effective. Nevertheless, there has not been wide uptake of routine EUS in PSC.

ASSESSING PORTAL HYPERTENSION, VARICES, AND BLEEDING RISK WITH EUS

Portal hypertension is the defining hemodynamic change in cirrhosis that is associated with the major complications of variceal bleeding, ascites, and encephalopathy^[60]. EUS can be used to diagnose splanchnic varices, predict the risk of bleeding, risk of recurrent bleeding, and guide therapeutic interventions (Figure 4)^[61]. In early reports, EUS was found to be inferior to conventional esophagogastroduodenoscopy (EGD) in detecting esophageal varices. Caletti *et al*^[62] compared EUS and conventional EGD findings in 40 patients with portal hypertension and 48 controls. The authors found a size-dependent sensitivity for EUS in detecting esophageal varices (14% for grade 1 varices *vs* 50% for grade 3 varices). Similarly, Burtin *et al*^[63] reported a sensitivity of 25% for grade 1 varices and 89% for grade 3 esophageal varices. However, more recent studies have shown EUS to be comparable to conventional EGD in detecting esophageal varices. In a study of 66 cirrhotic patients, EUS was able to detect esophageal varices in 48 (72%) patients compared to 49 (79%) detected by EGD^[64]. About half the patients in this study (31/66) had a previous episode of variceal bleeding which was treated by either band ligation or sclerosant injection. In a different study of 52 patients without a history of variceal bleeding, EUS was found to have a sensitivity of 96.4% when EGD was used as the gold standard^[65]. The improved diagnosis of esophageal varices with EUS over the years has been attributed to the use of a smaller echo-endoscope tip in newer echoendoscope models (which exerts less pressure on the varices) as well as a higher video resolution found in newer echo-endoscopes (and their respective processors).

Predicting risk of esophageal variceal recurrence

EUS has also been found to be helpful in predicting the risk of esophageal variceal recurrence after band ligation or sclerotherapy. In one study, 38 patients who underwent sclerotherapy for esophageal varices were followed with EUS every 3-4 mo for at least two years^[66]. The authors found that the risk of endoscopic variceal recurrence could be predicted by severe peri-esophageal collateral veins and large perforating veins of the esophagus, which in their study was seen on EUS as early as 3-4 mo prior to endoscopic variceal recurrence^[66]. In a study of 30 patients receiving endoscopic variceal ligation, a gastric cardiac perforating vein diameter greater than 3 mm was associated with a higher likelihood of recurrence of esophageal varices (90.9% *vs* 21.0%, $P < 0.01$)^[67]. In another study looking at EUS features before and after band ligation for a first esophageal variceal bleeding episode, presence of para-esophageal veins larger than 4 mm after band ligation was shown to predict variceal recurrence in 1 year with a sensitivity and specificity of 70.6% and 84.6%, respectively^[68]. In a prospective study of 45 patients who underwent band ligation for F2/F3 varices, the presence of severe peri-esophageal varices (defined as para-

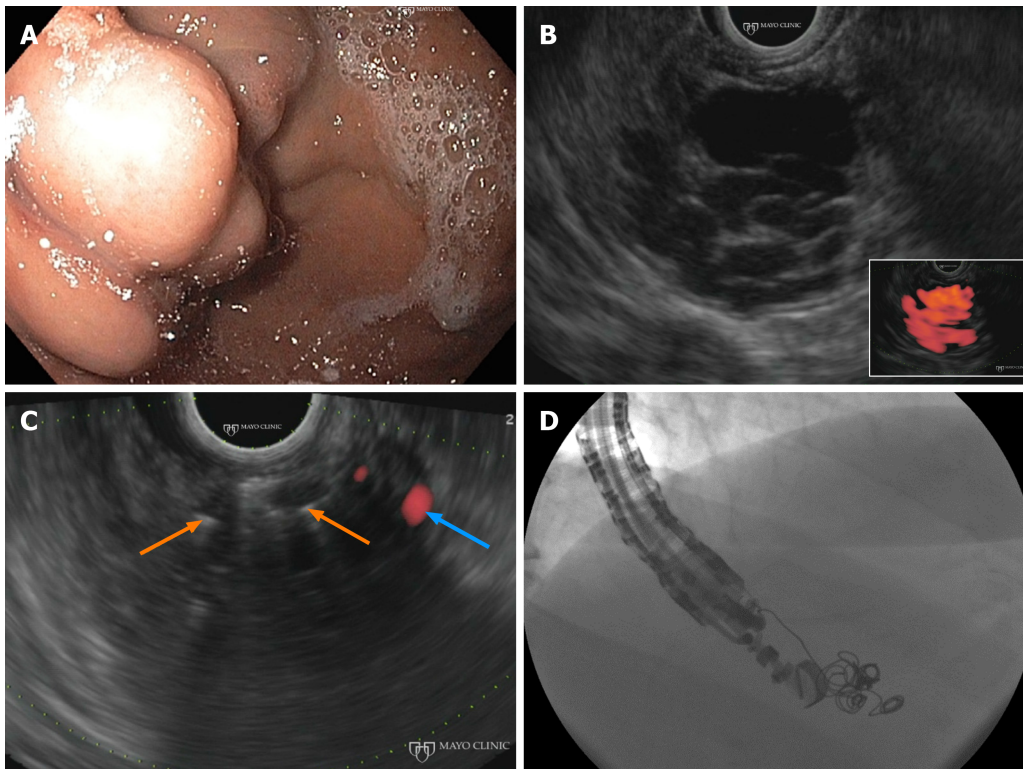


Figure 4 Endoscopic ultrasound-guided management of gastric varices. A: Gastric varices seen on endoscopy. B: Gastric varices appear anechoic on endoscopic ultrasound (EUS) grey-scale and are highlighted red by Doppler study (inset). C: Injection of embolization coils (orange arrows) into the varices results in near complete resolution of blood flow (blue arrow). D: Fluoroscopic visualization of EUS-guided coil embolization.

esophageal veins > 5 mm or peri-esophageal veins > 2 mm) and the presence of more than 5 esophageal collateral veins at baseline EUS were associated with a higher risk of variceal relapse in 1 year in a multivariate logistic regression analysis [odds ratio (OR) = 24.39; 95% confidence interval (CI): 2.34-253.78 and OR = 24.39; 95% CI: 2.34-253.78, respectively]. Of note, the reported confidence interval in this study was quite wide, likely due to the small sample size. High flow velocity in the left gastric vein and anterior branch dominant left gastric vein pattern also appear to be associated with a higher likelihood of esophageal recurrence in 1 year^[69].

Predicting risk of esophageal variceal bleeding

In addition to predicting risk of variceal recurrence, EUS may also predict the risk of recurrent variceal bleeding. A retrospective study of 306 patients who underwent endoscopic sclerotherapy for moderate to large or high-risk esophageal varices found that patients that had recurrent bleeding within one year had higher rates of detectable perforating veins and inflowing type of perforating veins prior to therapy, as well as higher rates of detection of cardiac intramural veins, perforating veins, and the inflowing type of perforating veins 3-5 mo post-endoscopic sclerotherapy^[70]. Another study found that the size of the diameter of para-esophageal veins (defined as veins external to the esophagus connecting to submucosal varices through perforating veins) was correlated with a higher rate of recurrent variceal bleeding^[71].

EUS-GUIDED TREATMENT OF VARICES

Considering the ability of EUS to identify para-esophageal and perforating veins which can contribute to esophageal variceal recurrence, it has been hypothesized that EUS-guided treatment of esophageal varices may reduce esophageal varices recurrence. However, a randomized clinical trial comparing traditional sclerotherapy and EUS-guided sclerotherapy of the feeding veins to esophageal varices did not show a lower recurrence rate for the EUS group^[72]. Additionally, no studies have compared EUS-guided therapy with band ligation for esophageal varices yet.

Unlike the case with esophageal varices, EUS appears to be significantly better than EGD in the detection and treatment of gastric varices, often thought to be more difficult to treat due to the inherent challenges with visualization of gastric varices.

Caletti *et al*^[62] demonstrated EUS was able to identify gastric varices (described as anechoic, circular structures beneath the submucosa) in 22 of 40 patients with portal hypertension while conventional EGD was only able to identify gastric varices in 10 of 40 patients. Several other studies have confirmed the superiority of EUS in the detection of gastric varices compared to conventional EGD with detection rates varying from 35% to 100%^[67,73,74].

Cyanoacrylate (CYA) glue injection has been used for the treatment of bleeding gastric varices due to its effectiveness and low risk of rebleeding^[75,76]. For this intervention, EUS has been used both as a confirmatory adjunct and as a real-time guide for the treatment of gastric varices^[77]. The presence of echogenic gastric varices and the absence of blood flow on doppler EUS can confirm the successful treatment of gastric varices after CYA injection, while the presence of blood flow in treated varices on follow up doppler EUS can suggest an increased risk of rebleeding^[78].

EUS can also be used to facilitate obliteration of gastric and ectopic varices using metallic coils^[79]. In a retrospective study, EUS-guided CYA injection and coil embolization were found to have similar rates of obliteration for primary and secondary prophylaxis of isolated gastric varices (IGV 1 and 2) with no patients having recurrent bleeding^[80]. The number of treatment sessions needed was fewer in patients receiving coil embolization (82% of patients had complete obliteration of a perforating vein after one session of coil embolization *vs* 53% after one session of CYA). Furthermore, of the 12 adverse events that occurred in this study, 11 occurred in the CYA group, with nine patients developing an asymptomatic pulmonary embolism, one with chest pain, and another had a fever. In the coil group, one patient developed bleeding from esophageal varices.

To reduce the risk of glue embolization, a combination of coil and glue obliteration of gastric varices has been proposed. In a study of 30 patients with active or recent gastric fundic varices (GOV-2 and IGV-1) who underwent EUS-guided coil embolization followed by 2-octyl-CYA glue injection, immediate hemostasis was achieved in all patients with an average of 1.4 mL of glue needed per patient with no procedure-related complications^[81].

CONCLUSION

EUS appears to be a relatively safe and effective diagnostic and therapeutic modality for many applications in patients with chronic liver disease. Compared with cross-sectional imaging, it has improved sensitivity for the identification of small liver lesions. It also allows for visualization and biopsy of the liver or lesions therein during the same session, potentially leading to earlier diagnoses. Despite previously reported difficulty with obtaining adequate tissue with earlier liver biopsy needles, newer generation needles appear to have largely overcome these earlier challenges. EUS also appears to be helpful in the evaluation of esophageal varices and the risk of future bleeding, as well as the treatment of gastric varices *via* glue injection or coil embolization. Lastly, EUS appears to be helpful in the diagnosis of indeterminate biliary strictures, though its application in this regard has remained relatively low. Given the strengths and advantages of EUS, it is expected that its clinical use and applications will grow.

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Case Control Study

Changing delta hepatitis patient profile: A single center experience in Valencia region, Spain

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Abstract

BACKGROUND

Delta hepatitis is a rare infection with an aggressive disease course. For almost three decades, however, there have been no epidemiological studies in our traditionally endemic area.

AIM

To investigate the prevalence of delta hepatitis in a sample of patients with chronic hepatitis B virus (HBV) infection followed at a Hepatology Unit in Valencia, Spain.

METHODS

Retrospective evaluation of anti-hepatitis D virus-immunoglobulin G seroprevalence among patients with chronic HBV infection ($n = 605$) followed at a reference Hepatology Unit in Spain.

RESULTS

The prevalence of anti-hepatitis D virus-immunoglobulin G among HBV-infected patients was 11.5%: Male (63%) and median age of 52 years. The majority were born in Spain (67%) and primarily infected through intravenous drug use. However, a significant percent (24.5%), particularly those diagnosed in more recent years, were migrants presumably nosocomially infected. Comorbidities such as diabetes (8.5%), obesity/overweight (55%), and alcohol consumption (34%) were frequent. A high proportion of patients developed liver complications such as cirrhosis (77%), liver decompensation (81%), hepatocellular carcinoma

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(HCC) (16.5%), or required liver transplantation (LT) (59.5%). Diabetes was associated with progression to cirrhosis, LT, and death. Male sex, increasing age, and alcohol were associated with LT and HCC. Compared to HBV mono-infected patients, delta individuals developed cirrhosis and liver decompensation more frequently, with no differences in HCC rates.

CONCLUSION

Patients infected in the 1980's were mostly locals infected through intravenous drug use, whereas those diagnosed recently are frequently non-Spanish natives from endemic areas. Regardless of their origin, patients are predominantly male with significant comorbidities, which potentially play a major role in disease progression. We confirm a high rate of subsequent liver complications.

Key words: Viral hepatitis; Delta hepatitis; Cirrhosis; Liver transplantation; Immigration; Valencia

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Core tip: Our study shows that there has been a change in the delta hepatitis virus-infected patient profile in our unit. Most patients infected in the 1980s, due to intravenous drug abuse, have progressed to cirrhosis and/or hepatocellular carcinoma and therefore still represent a significant burden on our Hepatology Unit. However, our main concern is with newly diagnosed patients, as there is a clear delay in the diagnosis of infection and cirrhosis. In addition, follow-up in their home countries has been poor. Most of them come from Eastern Europe and prior medical intervention was the main route of infection. We must also take into account the presence of comorbidities, *e.g.*, metabolic syndrome and alcohol intake, which may contribute to the aggressive progression of the disease. Therefore, we must devote our efforts to controlling these aspects and finding an effective treatment, given the poor results offered by pegylated- α -interferon.

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INTRODUCTION

Delta hepatitis is a rare infectious disease caused by hepatitis D virus (HDV), an RNA defective virus that requires the presence of hepatitis B surface antigen (HBsAg) to infect cells. Roughly 15 million people are infected worldwide. However, there are areas where the prevalence is higher, such as the Mediterranean basin^[1].

The importance of early detection of hepatitis B virus (HBV)-HDV coinfection is due to the aggressive course of the disease demonstrated in these patients compared to those only infected by HBV. At present, the only treatment available and recommended by Scientific Societies is alfa pegylated interferon^[2]. However, its use is restricted to highly selected patients (avoided in cirrhosis, active autoimmune disease and certain psychiatry disorders) and the response rate is low. Consequently, the only possible and effective way to control the spread of the infection currently is HBV vaccination.

In 1988, Buti *et al*^[3] reported a delta seroprevalence among HBV carriers of 15% in Spain; highlighting that intravenous drug users (IDUs) were the major risk group. Most recent studies from northern Spain, a non-traditional immigration area, have reported decreasing rates of infection over a 29-year period. A similar pattern has been documented in other European countries. The prevalence stabilized in the 1990s but was followed by a subsequent increase from 4.7% in 2003-2007 (lowest prevalence) to 7.4% in 2008-2012^[4].

There are no recent epidemiological data for our area, particularly information regarding the current profile of HDV infected patients. This information is especially needed given that the region is an immigration receiving area and the absence of

epidemiological information for almost three decades.

Based on this background, we aimed to update the epidemiological data on HDV infection in our geographical area. We specifically aimed to assess whether there had been changes in the profile of infected individuals, confirm the greater aggressive nature of HDV-HBV coinfection compared to HBV mono-infection, and describe risk factors for progression of disease.

MATERIALS AND METHODS

A retrospective single center study review of a database of HBV-infected patients followed in the Hepatology Unit of the Hospital Universitari I Politècnic “La Fe” (HUP La Fe). Inclusion criteria were: Diagnosis of delta hepatitis (confirmed by the presence of anti-HDV-immunoglobulin G [IgG]). No exclusion criteria were set.

The following data were extracted from the chart review: (1) Demographics including age, sex, country of birth, race, year of diagnosis, and first visit to the Unit, and probable mechanism of acquisition of HBV-HDV infection; (2) Presence of comorbidities such as diabetes mellitus, obesity, and alcohol intake, history of infection with hepatitis C virus (HCV) or human immunodeficiency virus (HIV); and (3) Hepatitis B e (HBe) antigen as well as alanine aminotransferase at diagnosis or first visit. In addition, the presence and time of occurrence of cirrhosis, liver failure, and hepatocellular carcinoma (HCC). The date a patient may have received a transplant or died was also recorded. The follow-up ended at the time of liver transplantation (LT), death or in the absence of these events, at the last visit.

The second part of the research was focused on the differences in disease evolution between HBV-HDV co-infected patients *vs* the HBV mono-infected patients. For this purpose, we designed a case-control study (1:1 match): HBV-infected patients to HBV-HDV-infected patients controlling for the year of diagnosis and sex. The following data were extracted: Presence of HCV or HIV coinfection, cirrhosis, liver decompensation, and liver cancer as well as laboratory tests gathered from the first visit onward including serum creatinine (mg/dL), estimated glomerular filtration rate (mL/min/1.73 m²), total bilirubin (mg/dL), albumin (g/dL), platelets (mm³), international normalized ratio, Child-Turcotte-Pugh, and Model for End-Stage Liver Disease staging.

Diagnostic criteria

HDV infection was defined as the presence of anti-HDV-IgG in patient serum. Chronic HBV infection was confirmed with the presence of HBsAg antigen in serum over a 6 mo period. Coinfection by HCV or HIV was confirmed with the presence of anti-HCV-IgG and anti-HIV-IgG, respectively. The diagnosis of cirrhosis was based on histologic, elastographic (liver stiffness greater than 12.5 kPas), and/or a composite of laboratory/imaging criteria^[5].

Statistical analysis

Continuous variables are expressed as the median and interquartile range, while categorical variables are depicted by the number and corresponding percentage. The comparison of qualitative variables was performed with χ^2 or Fisher's test. For the analysis of continuous variables with a normal distribution, the Student's *t*-test was used while the Mann-Whitney test was used for variables not following a normal distribution. The statistical significance was set at 0.05. To clarify the role of HDV coinfection in the development of the outcomes during the course of the disease previously mentioned, Kaplan-Meier survival analyses were done and the cumulative risk of developing these events was analyzed with Cox regression. All statistical tests performed for the analysis/comparison of variables excluded missing data. Particularly, patients missing one of the dates needed for the computation of a certain survival time outcome (depending on the risk being evaluated) were excluded for the estimation of the corresponding Kaplan-Meier curve.

The statistical analysis was performed using an SPSS program version 24.0 (IBM corporation). The R packages survival^[6] and survminer^[7] were used to produce the survival curves.

Confidentiality and data treatment

The study protocol coded MBH-INT-2018-01 was examined and approved by the Ethics Committee of HUP “La Fe”.

RESULTS

Description of the patient population

A total of 605 HBV-infected patients were included. Delta serology had not been tested in three cases. Seventy ($n = 70$) patients were anti-HDV-IgG positive (11.57%).

The general characteristics of the sample are shown in [Table 1](#). Patients were predominantly male (62.9%) and born in Spain (67.1%) with a median age of 52 years (interquartile range 12) at study entry. A non-negligible proportion (24.3%) were migrants from Eastern European countries. Regarding the route of infection, virtually half of the patients (45.7%) did not remember the possible source of the infection. Of the remaining patients 20% had a probable iatrogenic source of infection and (14.9%) had history of IDU.

Comorbidities

Although no patient was initially diabetic, diabetes developed over the course of the disease in a small proportion of individuals (8.6%). Furthermore, 55.5% were either obese or overweight with a median body mass index of 25.5 kg/m². Alcohol consumption was reported by roughly one-third of the sample (34.1%). Regarding coinfection with other viruses, 10.6% ($n = 7$) and 11.4% ($n = 8$) were HIV and HCV coinfecting, respectively. In most cases, coinfections occurred in Spanish patients with a history of IDU, infected during the peak incidence in the 1980s. Only 11.6% were HBe-antigen positive at diagnosis or first visit to the Unit. The majority (58.6%) of patients had alanine aminotransferase levels 1.5 times the upper limit of normality at diagnosis or first visit to the unit.

Treatment characteristics

One-third of the population (31.4%) had received pegylated interferon alfa therapy at some point in the disease course, with none responding. None of the patients was on interferon treatment at the time of the study. Early treatment discontinuation due to adverse events occurred in 20% of those treated. In contrast to delta therapy, antivirals against HBV were frequently used, with 54.3% of patients having received oral antivirals at some point during their disease course. Tenofovir was the most frequent antiviral used (36.8%) followed by Lamivudine (28.9%). Fifteen patients had never been treated with either pegylated interferon or oral antivirals.

Evolution events

Either at first visit or during follow-up, a large proportion (77.1%) of patients progressed to cirrhosis. Of these, the majority (80.9%) developed some type of decompensation, particularly ascites (71.1%) or upper gastrointestinal bleeding due to varices (26.3%). HCC developed in 16.9% of patients and liver transplantation was performed in 59.3%. Four patients died in the course of the follow-up, with two of the deaths related to their liver disease. Median age at time death was 50.5 years (interquartile range, 23).

Disease course

A survival analysis was performed to determine the cumulative risk of developing the different events during follow up ([Figure 1](#) and [Figure 2](#)). We differentiated the cumulative risk of developing cirrhosis and liver cancer according to whether they were diagnosed before or from 2000 ([Figure 3](#) and [Figure 4](#)). Progression to cirrhosis at 5, 10, 15, and 20 years from diagnosis occurred in 24%, 37%, 54%, and 73% of the patients, respectively. Liver cancer was observed at 5, 10, 15, and 20 years from diagnosis for the 5%, 5%, 8%, and 11%, respectively. In turn, the probability of developing decompensated cirrhosis from diagnosis reached 65%, 76%, 76%, and 88% at 5, 10, 15, and 20 years, respectively. Finally, LT-free mortality at 5, 10, 15, and 20 years from diagnosis was 3%, 3%, 7%, and 12%, respectively.

Factors associated with the development of the different events are shown in [Table 2](#). Diabetes was significantly associated with progression to cirrhosis, LT, and death. Furthermore, men were more frequently transplanted, with LT significantly increasing with patient age. Age was also significantly related to the onset of liver cancer. Alcohol consumption was also found to be associated with the need of LT as well as with liver cancer, but without reaching statistical significance in the latter. Finally, a history of interferon therapy was associated with a higher probability of LT.

Changes over time

We compared those patients diagnosed before the year 2000 to those diagnosed after 2000 to the present to assess the impact of changes in the Spanish population due to migration (based on data provided by the Immigration National Survey)^[8]. Significant differences were noticed regarding the time from diagnosis of infection to cirrhosis

Table 1 General characteristics (n = 70)

Demographic parameters		n (%)	Missing data, %
Sex, men		44 (62.9)	0
Country of birth by region			0
Spain		47 (67.1)	
Eastern Europe		17 (24.3)	
Other		6 (8.6)	
Probable route of infection			0
Unknown		32 (45.7)	
Iatrogenesis		14 (20.9)	
Intravenous drug use		10 (14.9)	
Household contact		6 (8.6)	
Sexual intercourse		4 (5.7)	
Blood transfusion		2 (2.9)	
Other		2 (2.9)	
Diabetes mellitus	Non-diabetic	64 (91.4)	0
Alcohol consumption		24 (34.3)	1.42
Weight			8.7
Underweight		2 (2.9)	
Normal weight		26 (37.1)	
Overweight		23 (32.9)	
Obese		12 (17.2)	
Serologic parameters			
HBe antigen	HBeAg+	8 (11.4)	1.4
HIV	Anti-HIV-IgG +	7 (10)	5.7
HCV	Anti-HCV-IgG +	8 (11.4)	0
Events			
Progression to cirrhosis		54 (77.1)	1.4
Liver decompensation		39 (72.2)	7.4
Most frequent type of liver decompensation			
Ascites		27 (71.1)	
Upper gastrointestinal bleeding		10 (26.3)	
Liver encephalopathy		1 (2.6)	
Hepatorenal syndrome		0 (0)	
Liver cancer		9 (12.9)	0
Liver transplant		32 (45.7)	0
Death		4 (5.7)	0

HBe: Hepatitis B e; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

among patients diagnosed before and after the year 2000 (Table 3). The median time interval from diagnosis to cirrhosis was 2 years [95% confidence interval (CI): 0.0-4.1] for those patients seen after the year 2000, while the interval was significantly longer 18.0 years (95%CI: 14.0-22.0); ($P < 0.0001$) for those patients seen before the year 2000.

Comparison between HBV mono-infected and HBV-HDV coinfecting patients

In an attempt to distinguish the effect of delta virus from that of HBV, HDV-HBV patients were paired with HBV-patients of the same sex and diagnosis year. Eventually, 62 HBV patients were included. Mono-infected HBV-patients were also predominantly men with a similar median age of 55.05 years (range: 30-83). Demographic, serological, and evolutive parameters of paired patients are summarized in Table 4. Cirrhosis and liver decompensation developed in a larger proportion of delta patients ($P < 0.000$). In fact, only 14 of HBV-mono-infected patients progressed to cirrhosis compared to 43 of those coinfecting. No differences in HCC development were found between groups.

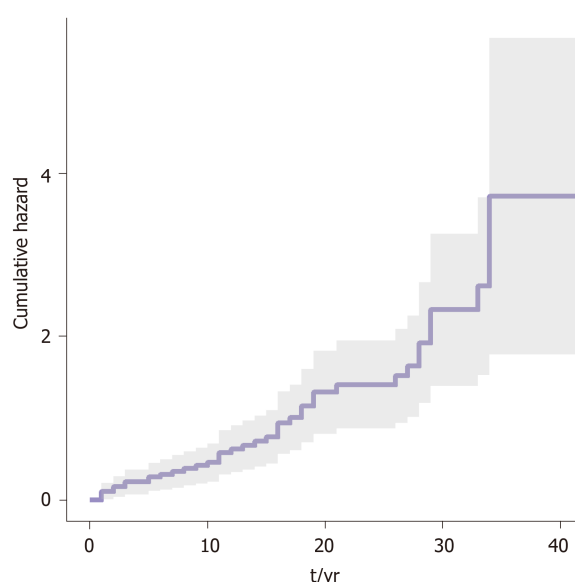


Figure 1 Cumulative risk of developing cirrhosis since diagnosis of infection.

DISCUSSION

The purpose of this study was to describe the prevalence and profile of the delta hepatitis-infected population in our Hepatology Unit over the last 30 years, and the differences due to migration. We also determined current risk factors associated with an unfavorable course and elucidated compliance with clinical practice guidelines.

The major findings can be summarized as follows. (1) Delta-infected patients were middle-aged males in whom HIV or HCV coinfection existed. Alcohol consumption and overweight-obesity were frequently present. (2) A substantial proportion (40%) was non-Spanish born, particularly those diagnosed more recently. (3) The profile of HDV-infected patients has substantially changed over the years, with a higher prevalence of Spanish-born, more frequently infected through IDU, and consequently more frequently coinfecting with either HIV or HCV among those diagnosed before the year 2000. By contrast, in those diagnosed more recently there was a significantly higher proportion of coinfecting immigrants, presumably due to nosocomial infection. (4) A significant proportion of patients progressed to cirrhosis and/or HCC, highlighting the aggressive nature of this co-infection. and (5) A relatively high number of patients (31.4%) had never received therapy with pegylated interferon.

Delta hepatitis is a concern in HBV-infected patients in our region. Indeed, the prevalence of HDV infection in our referral area is 11.57%, a rate higher than that previously reported both in our region as well as in surrounding countries. These findings might be due to our institution being a transplantation referral center and the recent migratory pattern. Recent studies in Spain have reported lower prevalence rates. According to Aguilera *et al*^[9], the delta prevalence in a tertiary hospital in Santiago de Compostela in 2018 was 4% among HBsAg+ patients followed since 2000. As in our study, patients diagnosed before 2000 were preferentially Spanish-born males, with a history of prior IDU. In fact, data from several studies have clearly shown that during the 1980 and 1990s, HDV-infected patients were mostly IDUs, with seroprevalence rates in IDUs ranging from 60% (de Miguel *et al*^[10]) to 68% (Castro Iglesias *et al*^[11]). In our region, a prevalence of 50% among IDUs was reported in the 1980s with no infections found among asymptomatic HBsAg+ carriers without a history of drug addiction^[12]. In the most recent study from 2017, the prevalence was 8.2% among 1215 patients with chronic HBV infection followed since 1983 in Northern Spain^[4]. This study also highlighted a decreasing prevalence among Spanish patients with the migrant population having become the main source of Delta infection nowadays. In addition, they detected an increased number of infections related to sexual practices. In our sample only 4 patients (3 women and 1 men) contracted the virus because of presumably “risky sexual practices,” but they were uniformly distributed between the two groups according to year of diagnosis, before and after 2000. Unfortunately, given the retrospective nature of this study, data on sexual practices were not universally recorded.

As previously reported, a proportion of patients were co-infected with other

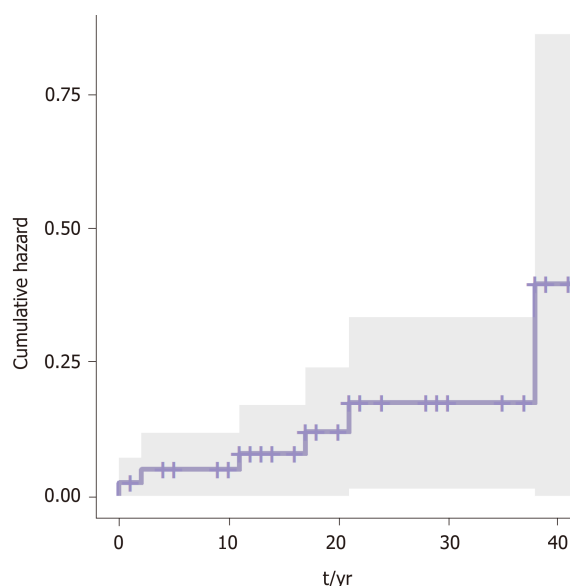


Figure 2 Cumulative risk of developing hepatocellular carcinoma since diagnosis of infection.

viruses. HIV (10.6%) and HCV (11.4%)-coinfection was mainly observed in the Spanish population, where the main risk factor was IDU. Interestingly, 5.7% of the sample had quadruple HBV, HDV, HCV, and HIV infection. As typically described in the Mediterranean area, most of the patients were anti-HBe positive at time of diagnosis.

The profile of currently diagnosed HDV-infected patients is that of middle-aged males, migrating from endemic areas, particularly Eastern Europe, where prior medical contact/intervention seems to be the main risk factor for infection. The few studies in our country evaluating the migrant population have solely focused on people from the African continent. López-Vélez *et al*^[13] found that 10% of HBsAg+ asymptomatic carriers from a cohort of sub-Saharan Africa immigrants residing in Madrid were also anti-HDV-IgG-positive. More recent data among immigrants from Equatorial Guinea living in Madrid demonstrated a prevalence of HDV infection of 20.9% of HBsAg carriers^[14].

Regarding the natural history of this infection, our data confirmed prior studies highlighting the aggressive course of HBV-HDV coinfecting patients. Indeed, three-quarters of patients progressed to cirrhosis and liver decompensation. Furthermore, only 3.3 years elapsed between the diagnosis of compensated cirrhosis and liver transplantation, death or last visit. The lack of response to pegylated interferon as well as the presence of frequent comorbidities, such as diabetes, obesity or alcohol intake may explain these findings. In summary, given the low efficacy rate of interferon, addressing adequately these comorbidities seems the best strategy to improve the long-term outcome of HDV-infected individuals. In addition, it is likely that the very short delay observed between diagnosis and cirrhosis in those diagnosed after the year 2000 may be related to the fact that most of these patients were migrants (46.7%) with no prior liver evaluation.

The main limitation of this study lies in its retrospective design. We cannot exclude selection bias potentially resulting in a prevalence underestimation and errors in estimating the time of infection. Furthermore, as this was a single center study, the sample size was relatively small which may have limited statistical power. Finally, no data on viral load was collected due to substantial missing data in early years and the change in quantitative techniques over time.

Despite these limitations, this research provides epidemiological data in a historically endemic area with new migration, highlighting changes in the patient profile, with a predominance of patients coming from Eastern Europe and the high frequency of comorbidities that may impact disease progression.

In conclusion, 11.5% of our HBV-infected population is coinfecting with HDV. Recently, diagnosed patients are non-Spanish born individuals coming from endemic countries where the main risk factor is presumably prior medical contact/intervention, inadequately diagnosed and followed until their arrival to Spain. Importantly, IDU patients infected during the first outbreak in the 1980s still represent a significant burden as most have progressed to cirrhosis and/or liver cancer.

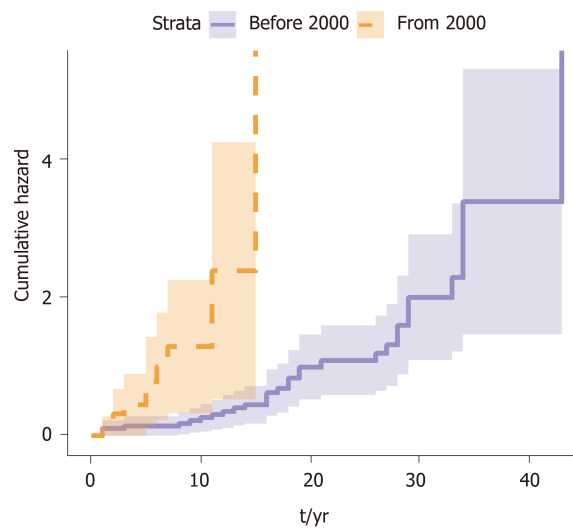


Figure 3 Cumulative risk of developing cirrhosis since delta hepatitis virus infection diagnosis for those patients diagnosed before and from 2000.

Independently from the country of origin, the majority of HDV-infected individuals are men with comorbidities, potentially playing a major role in disease progression. Finally, the low use of pegylated interferon together with its very low efficacy highlights the need for better therapies.

Table 2 Variables associated with the cumulative risk of different end-points since infection¹

	Cirrhosis	Liver cancer	Liver decompensation²	Transplantation	Survival
Age	+ (0.056) ²	++ (0.007)	NS	++ (0.034)	NS
Sex	NS	NS	+ (0.101)	++ (0.040)	NS
Country of birth	NS	NS	NS	NS	NS
Diabetes	++ (0.027)	NS	+ (0.085)	++ (0.013)	++ (0.004)
Overweight	NS	NS	NS	+ (0.104)	NS
Alcohol consumption	NS	+ (0.100)	NS	++ (0.019)	NS
HIV coinfection	NS	NS	NS	NS	NS
HCV coinfection	NS	NS	NS	NS	NS
Log [ALT]	NS	NS	NS	NS	NS
Treatment with IFN	NS	NS	NS	++ 0.034	NS
Antiviral treatment	+ (0.092)	NS	NS	NS	NS

¹>Except for clinical decompensation where baseline was “time of cirrhosis diagnosis”²For this purpose, we only took into consideration cirrhotic patients; ³Underlined values are those with a *P*-value < 0.1, because a tendency towards significance was observed. ALT: Alanine aminotransferase; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; IFN: Interferon; NS: No statistical significance.**Table 3** Differences according to year of diagnosis of infection, *n* (%)

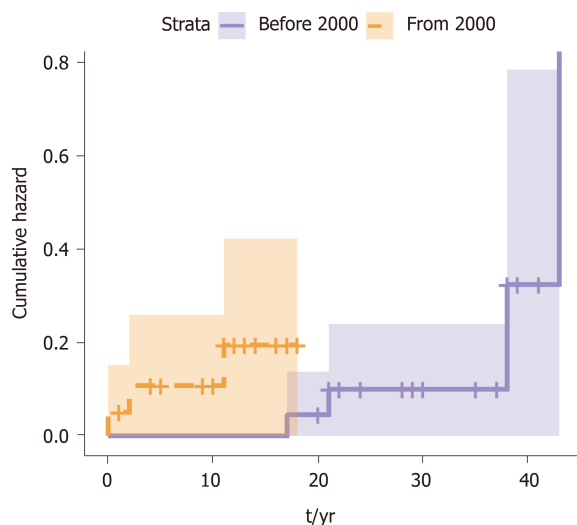
Demographic parameters		Total	Diagnosis before 2000	Diagnosis after 2000	<i>P</i> value
Country of birth by region					< 0.00
Spain		47 (67.1)	37 (92.5)	10 (33.3)	
Eastern Europe		17 (24.3)	3 (7.5)	14 (46.7)	
Other		6 (8.6)	0 (0)	6 (20)	
Diabetes mellitus	Diabetic	6 (8.6)	1 (2.3)	5 (16.7)	0.038
Alcohol consumption	Alcohol consumption	24 (34.3)	12 (30)	12 (41.4)	0.327
Weight					0.038
Underweight		2 (2.9)	1 (2.7)	1 (3.8)	
Normal weight		26 (37.1)	12 (32.4)	14 (53.8)	
Overweight		23 (32.9)	14 (37.8)	9 (34.6)	
Obese		12 (17.1)	10 (27)	2 (7.7)	
Serologic parameters					
HBe antigen	HBeAg +	8 (11.4)	8 (11.4)	0 (0)	0.008
HIV	Anti-HIV-IgG +	7 (10)	7 (10)	0 (0)	0.024
HCV	Anti-HCV-IgG+	8 (11.4)	8 (11.4)	0 (0)	0.009
Events					
Progression to cirrhosis		54 (77.1)	33 (82.5)	21 (72.4)	0.316
Liver decompensation		39 (72.2)	24 (77.4)	15 (75)	0.842
Liver transplant		32 (45.7)	22 (55)	10 (33.3)	0.74
Death		4 (5.7)	2 (5)	2 (6.7)	0.766

HBe: Hepatitis B e; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

Table 4 Descriptive characteristics of the paired samples of hepatitis B virus and hepatitis B virus-hepatitis D virus patients ($n = 62$), n (%)

		HBV patient sample	HDV patient sample	<i>P</i> value
Demographic parameters	Age in yr	55.05	48.90	< 0.024
	Sex, men	40 (64.5)	40 (64.5)	-
Serological parameters	HIV	0 (0)	7 (11.9)	< 0.005
	HCV	4 (6.5)	8 (12.9)	NS
Evolution parameters	Liver cirrhosis	14 (23.3)	43 (74.1)	< 0.000
	Liver decompensation	5 (8.2)	33 (57.9)	
	Liver cancer	4 (6.6)	5 (8.5)	NS

HBV: Hepatitis B virus; HCV: Hepatitis C virus; HDV: Hepatitis delta virus; HIV: Human immunodeficiency virus; NS: No statistical significance.

**Figure 4** Cumulative risk of developing hepatocellular carcinoma since hepatitis Delta virus infection diagnosis for those patients diagnosed before and after 2000.

ARTICLE HIGHLIGHTS

Research background

At present, epidemiological data and information regarding possible changes in the infected patient's profile are scarce. In addition, there were no updated epidemiological data in our region for three decades.

Research motivation

To carry out this research in Spain is of special interest given the idiosyncrasies of our country, and especially of our Region, Comunidad Valenciana, as an immigration receiving area.

Research objectives

The main objective was to describe the prevalence and profile of the hepatitis D virus (HDV) infection in our referral hospital. Secondary objectives were as follows: (1) To determine the epidemiology of the HDV infection; (2) To assess risk factors associated with this infection and determine whether the disease course is worse than in hepatitis B virus (HBV) mono-infected patients of the same age and sex; and (3) To elucidate whether the recommendations of the European guidelines for treatment are met.

Research methods

Retrospective evaluation of anti-HDV-immunoglobulin G seroprevalence among patients with chronic HBV infection followed in a reference Hepatology Unit. Demographic, clinical, analytic, serologic, and virology parameters were retrieved from chart review together with evolution events (cirrhosis, liver decompensation, liver transplantation, and death). HDV-HBV patients were matched 1:1 by age and year of diagnosis to HBV mono-infected patients.

Research results

Most of anti-HDV-IgG patients were men (63%) with a median age of 52 years. The majority

were Spaniards (67%), who were infected due to intravenous drug use; 24.5% of the population corresponded to migrants, presumably due to a nosocomial infection. Comorbidities (diabetes, alcohol consumption and overweight) were frequent in both groups. A high proportion of patients developed liver complications such as cirrhosis (77%), liver decompensation (81%), or HCC (16.5%), or required liver transplantation (59.5%). Compared to HBV mono-infected patients, delta individuals more frequently developed cirrhosis and liver decompensation, with no differences in HCC rates. The main limitation of this study lies in its retrospective quality. Therefore, we must base our conclusions on the current sample and follow patients prospectively in order to continue updating their data. Future research should collect virological data (HDV viral load) and determine the genotype in order to elucidate the role of these factors and determine if it is possible to ascertain individualized evolutionary courses according to the characteristics previously exposed.

Research conclusions

We confirmed our hypothesis that the HDV-infected population has changed starting in 2000 due to an increase in the immigrant population. However, the disease outcomes remain the same: Cirrhosis, impaired liver function and HCC. We have found a high prevalence of delta hepatitis in our area compared to other Spanish and European regions; probably because increased number of immigrants. This implies that we must be very diligent in screening for this infection in order to be able to offer patients early follow-up.

Research perspectives

Delta hepatitis remains a major concern and must follow these patients carefully given the significant complications seen on follow-up. Future research should elucidate the behavior of patients diagnosed over the last decade.

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Retrospective Cohort Study

Hospital teaching status on the outcomes of patients with esophageal variceal bleeding in the United States

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Author contributions: Patel P provided conception and design of the study, acquisition and analysis of data, and drafting of the manuscript; Rotundo L, Afridi F and Orosz E drafted the manuscript; Pyrsopoulos N contributed to the manuscript by providing revision and oversight of its writing.

Institutional review board

statement: This study did not require IRB approval due since the database is representative of nationally acquired data.

Informed consent statement: Due to the retrospective nature of this study as well as the using of a national database no human information was made available to authors. Therefore, informed consent was not needed to write this manuscript.

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Authors declare no conflict of interest for this article.

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Abstract

BACKGROUND

Acute variceal bleeding is a major complication of portal hypertension and is a leading cause of death in patients with cirrhosis. There is limited data on the outcomes of patients with esophageal variceal bleeding in teaching versus nonteaching hospitals. Because esophageal variceal bleeding requires complex management, it may be hypothesized that teaching hospitals have lower mortality.

AIM

To assess the differences in mortality, hospital length of stay (LOS) and cost of admission for patients admitted for variceal bleed in teaching versus nonteaching hospitals across the US.

METHODS

The National Inpatient Sample is the largest all-payer inpatient database consisting of approximately 20% of all inpatient admissions to nonfederal hospitals in the United States. We collected data from the years 2008 to 2014. Cases of variceal bleeding were identified using the International Classification of Diseases, Ninth Edition, Clinical Modification codes. Differences in mortality, LOS and cost were evaluated for patients with esophageal variceal bleed between teaching and nonteaching hospitals and adjusted for patient characteristics and comorbidities.

RESULTS

Between 2008 and 2014, there were 58362 cases of esophageal variceal bleeding identified. Compared with teaching hospitals, mortality was lower in non-teaching hospitals (8.0% vs 5.3%, $P < 0.001$). Median LOS was shorter in nonteaching hospitals as compared to teaching hospitals (4 d vs 5 d, $P < 0.001$). A

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higher proportion of non-white patients were managed in teaching hospitals. As far as procedures in nonteaching *vs* teaching hospitals, portosystemic shunt insertion (3.1% *vs* 6.9%, $P < 0.001$) and balloon tamponade (0.6% *vs* 1.2%) were done more often in teaching hospitals while blood transfusions (64.2% *vs* 59.9%, $P = 0.001$) were given more in nonteaching hospitals. Using binary logistic regression models and adjusting for baseline patient demographics and comorbid conditions the mortality, LOS and cost in teaching hospitals remained higher.

CONCLUSION

In patients admitted for esophageal variceal bleeding, mortality, length of stay and cost were higher in teaching hospitals versus nonteaching hospitals when controlling for other confounding factors.

Key words: Variceal bleeding; Teaching hospital; Mortality; National Inpatient Sample; Length of stay; Bleeding; Cirrhosis

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Core tip: This study assesses the outcomes of patients that present to the hospital with variceal bleeding amongst teaching and non-teaching hospitals. Patients that were managed at teaching facilities had higher mortality, length of stay and cost of hospitalization when compared to those at non-teaching facilities.

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INTRODUCTION

Acute variceal bleeding (AVB) as a direct consequence of portal hypertension remains the most lethal complication amongst cirrhotic patients. Over the past three decades, mortality due to variceal bleeding has steadily decreased in concurrence with improved endoscopic and pharmacological therapies, including endoscopic sclerotherapy, banding ligation, vasoactive agents, and antibiotic prophylaxis^[1-3]. In addition, a more efficient approach to improve hemodynamic stability, reduce portal pressure, and endoscopically treat variceal bleeding has now become the cornerstone of treatment in AVB^[3]. Prompt endoscopic therapy (≤ 12 h) for AVB has also been associated with better outcomes in cirrhotic patients^[4]. Early re-bleeding within 3-5 d of endoscopic therapy remains at approximately 20% for which aggressive treatment and early transjugular intrahepatic portosystemic shunt (TIPS) placement has shown increasing survival rates^[5]. Despite this progression in treatment modalities, bleeding from gastroesophageal varices maintains a 6-wk mortality in approximately 15%-20% of patients with underlying cirrhosis^[5]. Therefore, facilities to manage these cases require the necessary equipment and health-care personnel to initiate un-delayed treatment, prevent early re-bleeding, and decrease overall mortality.

It has been widely debated that certain disparities exist in delivery of medical care and patient safety outcomes when comparing teaching to nonteaching hospital^[6]. While earlier studies have reported higher quality care and better patient outcomes in teaching hospitals, others have considered this evidence unsubstantial^[6-10]. It has been further proposed that differences in patient outcomes within teaching versus nonteaching hospitals vary amongst multiple patient settings and specific diseases^[10]. For instance, one study has demonstrated that among common medical conditions, such as acute myocardial infarction and congestive heart failure, teaching hospitals have lower mortality rates when compared to non-teaching hospitals^[11]. Certain studies have shown that teaching hospitals may have higher rates of iatrogenic injury, though the underlying reasoning for this discrepancy has not been determined^[12]. Furthermore, with the enactment of resident work hour regulations for teaching hospitals, trends in patient safety outcomes and mortality have been highly scrutinized^[7,11]. Although duty hour limitations have been associated with a decrease

in overall mortality, data for patients admitted for gastrointestinal bleeding remained equivocal between hospital settings^[7].

An increasing amount of studies have focused on evaluating the most cost-effective manner in which to practice medicine. There has been an association between teaching hospitals and high-quality care because of their ability to provide specialized health care, perform advanced procedures, act as leaders in medical education and research, and offer care for the underserved populations^[7,8]. Within the current era of healthcare reform, most studies have shown higher costs in teaching hospitals compared with community facilities. This could be attributed to the utilization of more advanced, expensive diagnostic testing without any demonstrable improvement in outcomes^[13-15]. In regards to AVB, recent studies have suggested the average cost of in-hospital treatment to be \$6612 for those without any complications, and \$23207 for those with complications^[16]. After adjusting for geographic cost of living and patient factors, cost per case were similar across hospital type^[17].

Due to the complexity in management of esophageal variceal bleeding, one can hypothesize that teaching hospitals have lower mortality. Given the limited data on outcomes of patients with variceal bleeding in teaching versus nonteaching hospitals, our study aims to assess the differences in mortality, length of stay (LOS), and hospital costs for patients admitted for AVB among different hospital settings within the United States.

MATERIALS AND METHODS

Data source

The National Inpatient Sample (NIS), maintained by the Healthcare Cost and Utilization Project (HCUP) of the Agency for Healthcare Research and Quality, is the largest database of inpatient hospital stays in the United States^[18]. The NIS collects data from a 20% stratified sample of United States hospitals from 37 states and has been reliably used to estimate disease burden and outcomes. Each individual hospitalization is de-identified and maintained in the NIS as a unique entry with 1 primary discharge diagnosis and up to 29 secondary diagnoses during that hospitalization depending on the year of data collection. Each entry also carried information on patient demographics including age, sex, race, insurance status, primary and secondary procedures (up to 14), hospitalization outcome, total charges, and length of stay (LOS).

Study sample

The International Classification of Diseases 9th Version, Clinical Modification (ICD-9 CM) diagnosis codes (456.0 and 456.2) were used to identify patients (≥ 18 years) hospitalized with a primary diagnosis of esophageal variceal bleeding admitted between 2008 and 2014. If a patient had any other liver related diagnosis code ([Supplementary Table 1](#)) as their primary diagnosis they were also included. All patients that were admitted electively were excluded due to the inconsistency of elective admission and emergent nature of acute variceal bleeding. Only patients that had an EGD performed were included since endoscopy is necessary to diagnose a variceal bleed and also to exclude causes of nonvariceal upper GI bleeding. Cases that did not have mortality data or hospital teaching status were excluded. In total, 58362 cases were found using the above inclusion criteria. Secondary outcome variables were LOS and cost of hospitalization.

Hospital teaching status

Our primary exposure variable was the teaching status of the hospital each patient was treated at. In the NIS database, this data is divided into three separate categories: Rural, urban-nonteaching and urban teaching. For our study, the rural and urban-nonteaching categories were combined into one category termed non-teaching while the urban teaching category was used to delineate all teaching hospitals.

Predictive variables

Other variables that were studied included age (divided into three groups; < 40 years, 40-59 years and > 60 years), gender, race, primary payer, hospital location, hospital bed size, and transfer status (in from another acute care hospital *vs* not a transfer).

In order to assess for comorbidities, the data was extracted to include the Elixhauser comorbidity Index^[19]. This is a well-validated index based on ICD-9-CM codes that is meant to be used in large administrative data to predict mortality and hospital resource use^[20]. The index has 30 comorbid categories that include both liver disease and coagulopathy. Due to the nature of our primary diagnosis, we excluded both of these variables from our index and therefore studied only 28 comorbidities.

Since the NIS does not allow us to determine the severity of liver disease using either the Child-Pugh classification or Model for End Stage Liver Disease (MELD) score we assessed for separate conditions associated with liver decompensation. These included ascites (ICD-9-CM 789.5 and 789.59), hepatic encephalopathy (ICD-9-CM 572.2), spontaneous bacterial peritonitis (ICD-9-CM 567.23), hepatorenal syndrome (ICD-9-CM 572.4) and hepatocellular carcinoma (ICD-9-CM 155.0). Furthermore, we also separately analyzed data for patients with alcoholic cirrhosis (ICD-9-CM 571.2) as their underlying liver disease as this is one of the most common liver etiologies of variceal bleeding. Finally, common management options for esophageal variceal bleeding were also identified. These included blood transfusions (ICD-9-CM 99.00, 99.04, 99.05, 99.06, 99.07), balloon tamponade (ICD-9-CM 44.93 and 96.06), and portosystemic shunt (ICD-9-CM 39.1).

Statistical analysis

Hospital-level discharge weights provided by NIS were used to generate national estimates. Categorical variables were compared using the chi-square, whereas independent sample T test was used for continuous variables. Using binary logistic regression models mortality, LOS, and cost were examined after adjusting for baseline patient demographics, hospital details, procedures, and comorbid conditions. A *P*-value of < 0.05 was considered significant.

Inpatient cost of hospitalization was calculated by merging data from the NIS database with cost-to-charge ratios available from the Healthcare Cost and Utilization Project of the Agency for Healthcare Research and Quality. Given total charges for each inpatient stay available in the database, costs were then calculated by multiplying the total hospital charge with cost-to-charge ratios which were used to account for the inherent variability among hospitals and regions. All costs were adjusted for inflation according to the latest consumer price index data released by the United States government in December of 2017.

All analyses were performed using SPSS Statistics 23 (IBM Corp, Armonk, NY, United States).

RESULTS

Between 2008 and 2014 there were 58362 admissions for esophageal variceal bleeding that fit our inclusion criteria (7295 annually based on study period).

Teaching status of hospital

Amongst hospital admissions for esophageal variceal bleeding, a total of 30382 took place in teaching hospitals while 27979 took place in non-teaching hospitals. Demographics and hospital characteristics are provided in [Table 1](#). The average overall age of patients presenting with variceal bleeding was 55 (SD of 12) with a male predominance. More than half of the patients were Caucasian. The primary insurance payer was Medicare for a majority of the patients. A large portion of patients were treated in large hospitals (based on hospital region) and in the southern US. Though most patients were treated as initial admissions directly to the hospital under investigation, a small portion (7.4%) were transferred in from another acute care hospital.

In comparing teaching hospitals to non-teaching hospitals, the above characteristics remained true. Teaching hospitals had a higher percentage of minority patients compared to non-teaching hospitals. Teaching hospitals also had more Medicaid patients while non-teaching hospitals had more Medicare patients. A higher percentage of teaching hospitals with variceal bleeding was located in the Northeast and Midwest while a higher percentage of non-teaching hospitals was located in the South and the West. Furthermore, teaching hospitals had a greater percentage of transfers from outside acute care hospitals compared to non-teaching hospitals (11.5% *vs* 3.0% respectively).

Comorbid conditions as well as management for these patients can be seen in [Table 2](#). Amongst all patients, more than half had greater than or equal to three comorbid conditions other than their underlying liver disease as determined by the Elixhauser comorbidity index. Between group differences were not statistically significant however.

More importantly, teaching hospitals were more likely to admit patients with alcoholic cirrhosis (53.0% *vs* 50.6%), features of hepatic decompensation (ascites, hepatic encephalopathy), hepatorenal syndrome (4.2% *vs* 2.2%), and hepatocellular carcinoma (4.6% *vs* 2.5%) when compared to non-teaching hospitals.

In terms of management, non-teaching hospitals had a higher rate of transfusion (64.2% *vs* 59.9%) as compared to teaching hospitals and a lower rate of balloon

Table 1 Patient demographics and hospital characteristics

Variable	All (58362)	Teaching (30382)	Non-teaching (27979)	P value
Patient age (yr)				< 0.001
Median (SD)	55 (12.05)	54 (11.83)	55 (12.21)	
Sex				0.650
Female	18550 (31.8)	9601 (31.6)	8953 (32.0)	
Male	39803 (68.2)	20781 (68.4)	19026 (68.0)	
Race				< 0.001
White	35192 (60.3)	17166 (56.5)	18018 (64.4)	
Black	4202 (7.2)	2917 (9.6)	1287 (4.6)	
Hispanic	11030 (18.9)	5924 (19.5)	5092 (18.2)	
Asian or Pacific Islander	1167 (2.0)	668 (2.2)	532 (1.90)	
Native American	875 (1.5)	486 (1.6)	392 (1.40)	
Other	1751 (3.0)	1063 (3.5)	699 (2.50)	
Missing	4144 (7.1)	2188 (7.2)	1959 (7.0)	
Primary payer				< 0.001
Medicare	18442 (31.6)	8720 (28.7)	9709 (34.7)	
Medicaid	13540 (23.2)	7869 (25.9)	5680 (20.3)	
Private and HMO	14824 (25.4)	7960 (26.2)	6855 (24.5)	
Self-pay	7587 (13.0)	3767 (12.4)	3805 (13.6)	
No charge	700 (1.2)	425 (1.4)	308 (1.1)	
Other	3268 (5.6)	1671 (5.5)	1539 (5.5)	
Hospital bed size				< 0.001
Small	6245 (10.7)	4102 (13.5)	2182 (7.8)	
Medium	15408 (26.4)	8051 (26.5)	7386 (26.4)	
Large	36710 (62.9)	18229 (60.0)	18410 (65.8)	
Hospital region				< 0.001
Northeast	10564 (18.1)	6988 (23.0)	3581 (12.8)	
Midwest	9571 (16.4)	5621 (18.5)	3917 (14.0)	
South	23170 (39.7)	11636 (38.3)	11555 (41.3)	
West	15057 (25.8)	6137 (20.2)	8925 (31.9)	
Transfer in from acute care hospital				< 0.001
Yes	4319 (7.4)	3494 (11.5)	839 (3.0)	
No	54043 (92.6)	26888 (88.5)	27140 (97.0)	

tamponade (0.6% *vs* 1.2%) and portosystemic shunt (3.1% *vs* 7.9%). The *P* value was < 0.001 for all comparisons.

Mortality

The overall mortality for all patients presenting with esophageal variceal bleeding was 6.7% in our study population. The unadjusted mortality was higher in teaching hospitals when compared to non-teaching hospitals (8.0% *vs* 5.3% respectively, *P* < 0.001). Mortality was higher amongst patients that were black males, older than 60 years, admitted to a large hospital, and transferred from another acute care hospital. These findings are outlined in [Table 3](#).

Furthermore, there was also a significant difference amongst hospital teaching status and mortality when comparing comorbid conditions and management decisions ([Table 4](#)). There was a higher mortality in teaching hospitals in those patients with underlying alcoholic cirrhosis when compared to non-teaching hospitals (9.2% *vs* 6.3%) though this was not statistically different. The presence of liver decompensation (ascites and hepatic encephalopathy) was also associated with higher mortality in teaching hospitals compared to non-teaching hospitals. Hepatorenal syndrome and/or hepatocellular carcinoma also portended a higher risk for mortality in teaching hospitals. As far as management, mortality was higher in teaching hospitals when blood transfusions (8.7% *vs* 6.2%) or portosystemic shunts were performed (17.1% *vs* 9.9%) compared to non-teaching hospitals.

After adjustment for baseline patient characteristics including demographics, comorbid conditions, evidence of liver decompensation, management and transfer

Table 2 Patient comorbidities and management

Variable	All (58362)	Teaching (30382)	Non-teaching (27979)
Elixhauser comorbidities ^a			
0	2568 (4.4)	1398 (4.6)	1175 (4.2)
1	8929 (15.3)	4679 (15.4)	4281 (15.3)
2	14707 (25.2)	7565 (24.9)	7135 (25.5)
≥ 3	32157 (55.1)	16740 (55.1)	15388 (55.0)
Liver comorbidities			
Alcoholic cirrhosis	30232 (51.8)	16102 (53.0)	14157 (50.6)
Ascites	19551 (33.5)	10998 (36.2)	8534 (30.5)
Hepatic encephalopathy	9280 (15.9)	5286 (17.4)	4001 (14.3)
SBP	1109 (1.9)	760 (2.5)	336 (1.2)
Hepatorenal syndrome	1868 (3.2)	1276 (4.2)	616 (2.2)
Coagulopathy ^a	27255 (46.7)	14340 (47.2)	12870 (46.0)
Hepatocellular carcinoma	2101 (3.6)	1398 (4.6)	699 (2.5)
Management			
Blood transfusion	36184 (62.0)	18199 (59.9)	17963 (64.2)
Balloon tamponade	525 (0.9)	365 (1.2)	168 (0.6)
Portosystemic shunt	3268 (5.6)	2400 (7.9)	867 (3.1)

^a $P > 0.05$ in comparison between groups. All data are proportions [n (%)]. Comparisons between groups made with χ^2 test. All comparisons had $P < 0.001$ between groups unless otherwise stated.

status the only significant factors that were associated with higher mortality were gender, race, transfer in from outside hospital and teaching status of hospital. Males had a higher rate of mortality when compared to females with adjusted OR 1.271 (95%CI: 1.075-1.503) as did Blacks when compared to Whites with adjusted OR 1.607 (95%CI: 1.246-2.074). Patients that were transferred in from an acute care hospital had a higher mortality than those that did not with an adjusted OR 1.490 (95%CI: 1.172-1.894). Overall, the adjusted OR of mortality in teaching hospitals compared to non-teaching hospitals was 1.249 (95%CI: 1.066-1.463) (Table 3).

Length of stay and cost of hospitalization

The median length of stay for all centers was 4 d with an interquartile range (IQR) of 3-7. Teaching hospitals had a median LOS of 5 d while non-teaching hospitals had a LOS of 4 d. The cost of hospitalization overall was \$19049 in all centers. Teaching hospitals had a significantly higher cost of hospitalization of \$22355 compared to non-teaching hospitals at \$15535 (Table 5). Using linear regression analysis and controlling for baseline patient demographics, hospital characteristics, liver decompensation, associated conditions and management, the LOS and cost remained higher in teaching hospitals compared to non-teaching hospitals (Table 6).

DISCUSSION

Given the advancements in pharmacologic and endoscopic interventions, mortality rates have improved^[21]. Esophageal band ligation (EBL) has largely become standard of care due to its lower rate of complications, mortality and rebleeding^[22]. Though the rates of mortality due to acute variceal bleeding are steadily declining, this complication of portal hypertension still remains one of the leading causes of death in cirrhotic patients^[1]. Our study looked at 58362 cases of esophageal variceal bleeding in teaching and nonteaching hospitals between 2008 and 2014 and found that both mortality and length of stay was lower in non-teaching hospitals. In our hospitalized patient population, we found that a higher proportion of patients in teaching hospitals were of non-white race and underwent more rescue procedures (portosystemic shunt insertion). However, blood transfusions were more commonly given in nonteaching hospitals.

While upper gastrointestinal bleeding has previously been associated with a high mortality (up to 10%)^[23,24], recent studies have shown that the mortality rate has decreased over the past 20 years to as low as 2.1%^[21]. Additionally, rates of EGD performed early in the hospital stay have been steadily increasing over the same time

Table 3 Mortality associated with patient demographics and hospital characteristics

Variable	Mortality (%) ^a	Unadjusted OR (95%CI)	Adjusted OR (95%CI) [†]	P value
Patient age (yr)				0.226
Age < 40	5.5%	1.0	1.0	
Age 40-59	6.7%	1.226 (0.900-1.669)	1.182 (0.858-1.628)	0.196
Age > 60	7.1%	1.312 (0.955-1.801)	1.397 (0.989-1.974)	0.094
Sex				0.001
Female	5.6%	1.0	1.0	
Male	7.3%	1.323 (1.126-1.555)	1.271 (1.075-1.503)	0.005
Race				0.001
White	6.4%	1.0	1.0	
Black	10.2%	1.664 (1.308-2.118)	1.607 (1.246-2.074)	< 0.001
Hispanic	6.1%	0.948 (0.778-1.155)	0.942 (0.766-1.157)	0.567
Asian or Pacific Islander	6.6%	1.040 (0.621-1.742)	1.046 (0.619-1.768)	0.867
Native American	6.7%	1.062 (0.587-1.922)	1.071 (0.572-2.002)	0.831
Other	7.6%	1.214 (0.971-1.518)	1.131 (0.895-1.429)	0.304
Primary payer				0.001
Medicare	6.7%	1.0	1.0	
Medicaid	7.9%	1.180 (0.978-1.423)	1.027 (0.844-1.250)	0.789
Private and HMO	6.0%	0.887 (0.729-1.078)	0.837 (0.683-1.026)	0.087
Self-pay	7.2%	1.069 (0.849-1.346)	1.098 (0.864-1.395)	0.445
No charge	4.0%	0.580 (0.254-1.326)	0.517 (0.214-1.247)	0.142
Other	4.9%	0.706 (0.487-1.025)	0.671 (0.456-0.988)	0.043
Hospital bed size				0.001
Small	5.2%	1.0	1.0	
Medium	5.8%	1.115 (0.836-1.486)	1.094 (0.814-1.470)	0.551
Large	7.4%	1.458 (1.123-1.892)	1.272 (0.970-1.668)	0.082
Hospital region				0.143
Northeast	7.4%	1.0	1.0	
Midwest	7.1%	0.961 (0.760-1.216)	0.960 (0.744-1.238)	0.752
South	6.1%	0.814 (0.667-0.994)	0.914 (0.740-1.129)	0.403
West	7.0%	0.946 (0.766-1.169)	1.110 (0.882-1.397)	0.372
Transfer in from acute care hospital				< 0.001
No	6.2%	1.0	1.0	
Yes	11.9%	2.041 (1.643-2.536)	1.490 (1.172-1.894)	0.001
Hospital teaching status				< 0.001
Teaching	8.0%	1.557 (1.345-1.803)	1.249 (1.066-1.463)	0.006
Non-teaching	5.3%	1.0	1.0	

^aP < 0.001 for all comparisons of crude mortality between groups in bivariate χ^2 analyses.[†]Adjusted for age, sex, race, primary insurance, hospital bed size, region, transfer status, features of hepatic decompensation, and management.

period^[21]. Interventions that are performed during EGD to limit bleeding, likely explain the reductions in mortality, transfusions and need for further supportive therapies such as vasopressors or ICU stays. Delays in EGD in nonteaching hospitals, may therefore, explain the higher rates of transfusions in those institutions but not the decreased mortality.

Our study revealed that hospital costs were higher in teaching hospitals. Prior studies have found that the overall cost during the hospitalization, including the cost of any procedures, were higher in patients who did not undergo early EGD^[25].

A main strength of our study is that our sample size is representative of the inpatient population throughout the United States. It is unique in that it looks at the differences among teaching versus nonteaching hospitals in a study population that is nationally representative. Our study period was recent, from 2008 to 2014, and thus reflective of recent endoscopic management for esophageal variceal bleeds.

The current study has several limitations. First, the NIS is a database reliant on the delineation and coding of medical diagnoses, which if performed incorrectly can

Table 4 Patient comorbidities and management with associated mortality

Variable	Mortality overall (n = 58362)	Mortality teaching (n = 30382)	Mortality non-teaching (n = 27979)
Elixhauser comorbidities			
0	4202 (7.2)	3008 (9.9)	1455 (5.2)
1	4085 (7.0)	2734 (9.0)	1287 (4.6)
2	4144 (7.1)	2886 (9.5)	1371 (4.9)
≥ 3	3794 (6.5)	2157 (7.1)	1567 (5.6)
Liver comorbidities			
Alcoholic cirrhosis ^a	4494 (7.7)	2795 (9.2)	1763 (6.3)
Ascites	5953 (10.2)	3585 (11.8)	2266 (8.1)
Hepatic encephalopathy	9338 (16.0)	5165 (17.0)	3973 (14.2)
SBP	11322 (19.4)	8294 (27.3)	1567 (5.6)
Hepatorenal syndrome	23695 (40.6)	12335 (40.6)	11415 (40.8)
Coagulopathy	4844 (8.3)	2917 (9.6)	1931 (6.9)
Hepatocellular carcinoma	7821 (13.4)	4770 (15.7)	2266 (8.1)
Management			
Blood transfusion	4319 (7.4)	2643 (8.7)	1735 (6.2)
Balloon tamponade	30290 (51.9)	15616 (51.4)	14857 (53.1)
Portosystemic shunt	8871 (15.2)	5195 (17.1)	2770 (9.9)

^a $P > 0.05$ in comparison between groups. All data are proportions [n (%)]. Comparisons between groups made with χ^2 test. All comparisons had $P < 0.001$ between groups unless otherwise stated.

predispose to classification errors and inaccuracies. Second, a patient's clinical acuity, preoperative and intraoperative performance status, and endoscopic procedure findings cannot be accessed within the NIS^[26]. Third, the inherent features of the database do not allow us to fully assess a patient's hospital course. This further limits our ability to distinguish temporal relationships between medical diagnoses and their causality with patient outcomes. Fourth, we did not include patients diagnosed with an AVB after admission to the hospital, which could have underestimated rates of mortality. Fifth, we were also unable to discern rates of re-bleeding post endoscopic intervention or if these AVB events were primary or recurrent, which if recurrent would place a patient at a higher risk of mortality^[27,28]. Moreover, pertinent variables including lab values, endoscopic findings and therapies, vital signs were missing as these are not available using the NIS database. Lastly, pharmacological therapy such as octreotide as well as prophylaxis measures with nonselective beta-blockers is not included in the NIS, which are important confounders that may have affected patient outcome between hospital settings.

Despite this, the findings are intriguing. Further prospective studies may need to be completed in order to determine causality and delineate whether teaching status affects patient outcomes.

Table 5 Length of stay and cost of hospitalization

Variable	All (58362)	Teaching (30382)	Non-teaching (27979)	P value
Length of stay				< 0.001
Median (IQR) in days	4 (3-7)	5 (3-8)	4 (3-6)	
Cost of hospitalization				< 0.001
Mean in US dollars (SD)	\$19049 (11880)	\$22355 (12996)	\$15535 (10935)	

IQR: Interquartile range.

Table 6 Mortality, length of stay and cost in teaching vs nonteaching logistic regression

Variable	OR/coefficient	95%CI	P value
Mortality	1.249	1.066-1.463	0.006
LOS	1.72	1.46-1.97	< 0.001
Cost	6651	5646-7656	< 0.001

LOS: Length of stay; OR: Odds ratio.

ARTICLE HIGHLIGHTS

Research background

Acute variceal bleeding is a major complication of portal hypertension and is a leading cause of death in patients with cirrhosis. There is limited data on the outcomes of patients with esophageal variceal bleeding in teaching versus nonteaching hospitals.

Research motivation

To understand if the teaching status of a hospital has better or poorer outcomes in management of patients with variceal bleeding.

Research objectives

Compare outcomes of mortality, length of stay and cost of hospitalization amongst patients presenting with acute variceal bleeding in cohorts of teaching vs nonteaching hospitals.

Research methods

We looked at retrospective data from a large national database of patients that presented with acute variceal bleeding.

Research results

The mortality, length of stay and cost of hospitalization was higher amongst patients with acute variceal bleeding that presented to a teaching hospital. When controlling for comorbidities and hospital characteristics this remained statistically significant.

Research conclusions

Teaching hospitals did worse in outcomes for patients with variceal bleeding when compared to non-teaching hospitals. Further details may need to be deciphered as to what could contribute to these findings.

Research perspectives

Prospective studies at teaching and non-teaching institutions when controlling for severity of illness can shed light on whether teaching hospitals need to improve their delivery of care for patients with variceal bleeding.

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Retrospective Cohort Study

LIV-4: A novel model for predicting transplant-free survival in critically ill cirrhotics

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Abstract

BACKGROUND

Critically ill patients with cirrhosis, particularly those with acute decompensation, have higher mortality rates in the intensive care unit (ICU) than patients without chronic liver disease. Prognostication of short-term mortality is important in order to identify patients at highest risk of death. None of the currently available prognostic models have been widely accepted for use in cirrhotic patients in the ICU, perhaps due to complexity of calculation, or lack of universal variables readily available for these patients. We believe a survival model meeting these requirements can be developed, to guide therapeutic decision-making and contribute to cost-effective healthcare resource utilization.

AIM

To identify markers that best identify likelihood of survival and to determine the performance of existing survival models.

METHODS

Consecutive cirrhotic patients admitted to a United States quaternary care center ICU between 2008-2014 were included and comprised the training cohort. Demographic data and clinical laboratory test collected on admission to ICU were analyzed. Area under the curve receiver operator characteristics (AUROC) analysis was performed to assess the value of various scores in predicting in-

protections to maintain the data in a secure manner with access limited to the study team and if sharing or releasing identifiable data to any outside person or entity will not occur. For this reason, no Informed Consent Form was used for this study.

Conflict-of-interest statement:

There are no conflicts of interest associated with any of the senior authors or other coauthors who contributed their efforts to this manuscript. All the authors have no conflict of interest related to the manuscript.

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hospital mortality. A new predictive model, the LIV-4 score, was developed using logistic regression analysis and validated in a cohort of patients admitted to the same institution between 2015-2017.

RESULTS

Of 436 patients, 119 (27.3%) died in the hospital. In multivariate analysis, a combination of the natural logarithm of the bilirubin, prothrombin time, white blood cell count, and mean arterial pressure was found to most accurately predict in-hospital mortality. Derived from the regression coefficients of the independent variables, a novel model to predict inpatient mortality was developed (the LIV-4 score) and performed with an AUROC of 0.86, compared to the Model for End-Stage Liver Disease, Chronic Liver Failure-Sequential Organ Failure Assessment, and Royal Free Hospital Score, which performed with AUROCs of 0.81, 0.80, and 0.77, respectively. Patients in the internal validation cohort were substantially sicker, as evidenced by higher Model for End-Stage Liver Disease, Model for End-Stage Liver Disease-Sodium, Acute Physiology and Chronic Health Evaluation III, SOFA and LIV-4 scores. Despite these differences, the LIV-4 score remained significantly higher in subjects who expired during the hospital stay and exhibited good prognostic values in the validation cohort with an AUROC of 0.80.

CONCLUSION

LIV-4, a validated model for predicting mortality in cirrhotic patients on admission to the ICU, performs better than alternative liver and ICU-specific survival scores.

Key words: Risk stratification; Resource allocation; Intensive care unit; Acute-on-chronic liver failure; Modeling; Mortality

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Core tip: Critically ill patients with cirrhosis have higher mortality rates in the intensive care unit (ICU) than patients without chronic liver disease. None of the currently available prognostic models have been widely accepted for use in cirrhotic patients in the ICU, perhaps due to complexity of calculation. We believe survival modeling can guide therapeutic decision-making and contribute to cost-effective healthcare resource utilization. We describe the development of a novel model to predict in-hospital mortality in critically ill patients with cirrhosis. Our validated model for predicting mortality on admission to the ICU performs better than previously published liver and ICU-specific scores.

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INTRODUCTION

Patients with cirrhosis, particularly those with acute decompensation necessitating intensive care unit (ICU) admission, are at elevated risk for short-term mortality^[1-3]. Acute-on-chronic liver failure, as defined by sequential organ failure in patients with cirrhosis, portends a poorer prognosis, with 28-day mortality approaching 80% in patients with 3 or more organ failures^[1,4-7]. The most recent data from the nationwide inpatient sample in the United States estimates that more than 26000 patients with cirrhosis are admitted to ICUs annually, of which less than half (about 47%) survive hospitalization^[7,8]. Critical care for patients with cirrhosis is estimated to cost upwards of United States \$3 billion annually, with each admission totaling on average United States \$116200^[8]. Survival analysis tools aid in the early identification of critically ill

patients, which, when applied as part of therapeutic decision-making, can help guide goals of critical care discussions with patients and their families, and may contribute to cost-effective healthcare resource utilization^[9].

To this end, the Acute Physiology and Chronic Health Evaluation (APACHE) methodology^[10] and the Simplified Acute Physiology Score (APS)^[11] are widely applied to estimate the risk of inpatient mortality based on values collected from within the first 24 hours of critical care admission. Similarly, the Sequential (or Sepsis-Related) Organ Failure Assessment (SOFA) is commonly used to describe, compare, and track a patient's clinical course in the ICU^[9]. On the other hand, liver-specific scores, such as the Child-Pugh Score, Model for End-Stage Liver Disease (MELD), MELD-Sodium (MELD-Na) and Chronic Liver Failure-SOFA (CLIF-SOFA) are broadly applied in patients with liver disease to predict 90-day mortality, allocate donor organs for liver transplantation, and to define hepatic decompensation as well as acute-on-chronic liver failure^[1,12-16]. Critical care scoring systems that include the assessment of organ dysfunction have generally performed as well, or better, in patients with cirrhosis than these liver-specific models for short-term mortality^[1,17-28]. However, none of these prognostic models have been widely accepted for use in clinical practice, perhaps due to complexity of calculation, or lack of universal variables readily available for cirrhotic patients in the ICU. We aimed to identify markers that best identify likelihood of transplant-free survival in critically ill patients with cirrhosis and to determine the performance of existing survival models.

MATERIALS AND METHODS

Study aims

The aims of this study are to: (1) Identify clinical and laboratory markers universally available at the time of ICU admission that best identify the likelihood of survival; and (2) To compare this model to existing survival models.

Study design

Patients over the age of 18 with a diagnosis of cirrhosis admitted between 2008-2014 to an ICU at a major quaternary referral and liver transplantation center in the United States comprised the training cohort. Patients from the APACHE IVb database (a prospective database of consecutive patients admitted to the ICU) were identified retrospectively by searching the database for the APACHE chronic health items (1) hepatic failure and (2) cirrhosis. The diagnosis of cirrhosis was subsequently confirmed either (1) radiographically, based on imaging evidence of cirrhosis or portal hypertension; (2) histologically by liver biopsy, if performed, and/or (3) by evidence of hepatic decompensation, including hepatic encephalopathy, variceal bleeding, or ascites. Patients with acute liver failure, history of liver transplantation, or who underwent liver transplantation during the contemporaneous hospital admission were excluded from the analysis.

Patient population

Demographic patient data consisting of age, gender, co-morbidities, etiology of chronic liver disease, and vital signs on admission to ICU were recorded from the electronic medical record. Clinical laboratory tests collected on admission to ICU included platelet count, prothrombin time (PT), International normalized ratio, lactate, arterial blood gas, pH, partial arterial pressure of carbon dioxide and oxygen, inspired oxygen concentration (FiO₂), oxygen/FiO₂, alveolar-arterial partial pressure oxygen gradient (A-a gradient), hematocrit, white blood cell count (WBC), potassium, blood urea nitrogen, albumin, sodium (Na), creatinine, bilirubin, bicarbonate, and glucose. Additional clinical parameters, including 24-hour urine output, need for mechanical ventilation, need for dialysis, variceal hemorrhage, Glasgow coma scale, vasopressor dose, and degree of ascites and encephalopathy were recorded. This information was used to grade the severity of liver disease and prognosticate ICU mortality based on the calculation of previously validated liver-specific and ICU prognostic scores, including the MELD, MELD-Na, Child-Pugh, SOFA, CLIF-SOFA, Royal Free Hospital (RFH), APS and APACHE III scores. Subjects were followed from admission to hospital discharge or death.

The internal validation cohort was comprised of prospectively enrolled patients over the age of 18 with a diagnosis of cirrhosis admitted to the same institution as the training cohort between 2015-2017 and were subject to identical exclusion criteria. All patients that met the inclusion criteria were included in the analysis; no formal sample size calculations were done. The Institutional Review Board of the Cleveland Clinic Foundation reviewed and approved this study. On behalf of all authors, the

corresponding author states that there is no conflict of interest.

Statistical analysis

A univariate and then multivariate analysis was performed to assess factors associated with in-hospital mortality. Data are presented as mean \pm standard deviation, median (25th, 75th percentiles) or *n* (%). Analysis of variance or the non-parametric Kruskal-Wallis tests were used for continuous or ordinal variables and Pearson's chi-square tests were used for categorical factors. In addition, Spearman correlations coefficients were used to assess correlation between length of stay and the different scores.

Receiver Operating Characteristics (ROC) analysis was performed to assess the value of various scores in predicting in-hospital mortality; areas under the ROC curves (AUROC) and corresponding 95% confidence intervals are presented.

A predictive model was developed using logistic regression analysis. An automated stepwise variable selection method performed on 1000 bootstrap samples was used to choose the final model. All variables known at time of ICU admission were considered for inclusion. Variables with inclusion rates of at least 50% were further assessed and the most parsimonious model with highest AUROC is reported. Variable transformations were assessed to account for any possible non-linearity. Observations with missing values were not included when building models.

After choosing the final model, the method described by Harrell^[29] was used to compute the validation metric with over-fitting bias correction through bootstrap resampling. A thousand bootstrap samples (*B* = 1000) were drawn from the original data set and a new model with the same model settings was built on each bootstrap resample. Prediction on patients that were not chosen in the resample was calculated. An optimism factor was calculated over the 1000 new models and the bias-corrected validation metric was obtained by subtracting this optimism value from the AUROC directly measured from the original model. In addition, the Hosmer-Lemeshow goodness-of-fit χ^2 test and calibration plots were used to assess calibration of the models. DeLong's method was used to compare predictive ability of LIV-4 to that of the various scores by comparing AUROCs^[30]. A univariable analysis was performed to assess differences between the training and validation cohorts. SAS (version 9.4, The SAS Institute, Cary, NC, United States) was used for all analyses and a *P* < 0.05 was considered statistically significant. The statistical review was performed by a biomedical statistician.

RESULTS

Training cohort

Patient characteristics: Training Cohort. In total, 436 patients cirrhotic patients, aged 57 ± 10.6 years, 65.4% males, mostly with alcohol-related liver disease -(45.2%), Hepatitis C Virus -(33.7%) and Non-alcoholic steatohepatitis -(22%) related cirrhosis were included in the training cohort (Table 1). The majority of patients presented with severely decompensated liver disease, evidenced by the presence of moderate/severe encephalopathy (47.5%), moderate/severe ascites (44.3%), or variceal bleeding (25.7%) on admission, with median MELD score of 23.3 and Child-Pugh Score of 10.2 (C). 119 patients (27.3%) died in the hospital. The median ICU length of stay was 2.6 (25th, 75th percentiles: 1.4, 5.2) d and the median hospital length of stay was 8.7 (4.7, 16.8) d.

Factors associated with in-hospital mortality

Table 1 summarizes univariable comparisons of subjects who died and those who were discharged alive. There was no significant difference in patient age, gender, ethnicity, etiology of liver disease or co-morbidities between survivors and non-survivors. Survivors had lower MELD (20.3 *vs* 31.1) and Child-Pugh (10.3 *vs* 11.9) scores. Variceal hemorrhage (*P* = 0.26), presence/grade of hepatic encephalopathy (*P* = 0.43), and presence/degree of ascites (*P* = 0.85) were not predictive of in-hospital mortality.

Patients who died in the hospital were more likely to require mechanical ventilation (49.6% *vs* 35%, *P* = 0.005) and dialysis (12.6% *vs* 6.3%, *P* = 0.031) on admission to the ICU than patients who survived. Patients who did not survive hospitalization had significantly lower mean arterial pressure (MAP), temperature and Glasgow coma scale (*P* < 0.001). Additionally, non-survivors were more likely to have lower hematocrit and bicarbonate, as well as higher WBC, A-a gradient, lactate, PT/International normalized ratio, potassium, blood urea nitrogen, creatinine, and bilirubin (*P* < 0.001). There was no significant difference in serum sodium or albumin levels between survivors and non-survivors (*P* = 0.81 and 0.57, respectively).

Table 1 Training cohort: Patient characteristics and univariate analysis of factors associated with In-hospital mortality

Factor	Total (n = 436)		Discharged alive (n = 317)		In-hospital death (n = 119)		P value
	n	Summary	n	Summary	n	Summary	
Age (yr)	436	57.0 ± 10.6	317	57.5 ± 10.3	119	55.5 ± 11.3	< 0.081 ¹
Gender	436		317		119		< 0.62 ³
Female		151 (34.6)		112 (35.3)		39 (32.8)	
Male		285 (65.4)		205 (64.7)		80 (67.2)	
Ethnicity	420		308		112		< 0.41 ³
White/Caucasian		340 (81.0)		254 (82.5)		86 (76.8)	
Black/African/Haitian		60 (14.3)		41 (13.3)		19 (17.0)	
Other		20 (4.8)		13 (4.2)		7 (6.3)	
Any previous ICU stay during same admission	436	13 (3.0)	317	6 (1.9)	119	7 (5.9)	< 0.029 ³
Comorbidities							
Diabetes	436	129 (29.6)	317	100 (31.5)	119	29 (24.4)	< 0.14 ³
COPD	436	61 (14.0)	317	49 (15.5)	119	12 (10.1)	< 0.15 ³
Severe COPD	436	12 (2.8)	317	10 (3.2)	119	2 (1.7)	< 0.40 ³
Solid tumor with metastasis	436	3 (0.69)	317	1 (0.32)	119	2 (1.7)	< 0.18 ²
Immune suppression	436	21 (4.8)	317	16 (5.0)	119	5 (4.2)	< 0.71 ³
Mechanical ventilation	436	170 (39.0)	317	111 (35.0)	119	59 (49.6)	< 0.005 ³
Dialysis > 2 times in 7 d	436	35 (8.0)	317	20 (6.3)	119	15 (12.6)	< 0.031 ³
Liver disease etiology							
AIAT	436	9 (2.1)	317	8 (2.5)	119	1 (0.84)	< 0.27 ³
AIH	436	17 (3.9)	317	11 (3.5)	119	6 (5.0)	< 0.45 ³
ALD	436	197 (45.2)	317	138 (43.5)	119	59 (49.6)	< 0.26 ³
Cryptogenic	436	25 (5.7)	317	21 (6.6)	119	4 (3.4)	< 0.19 ³
HCV	436	147 (33.7)	317	102 (32.2)	119	45 (37.8)	< 0.27 ³
HBV	436	9 (2.1)	317	5 (1.6)	119	4 (3.4)	< 0.24 ³
NASH	436	96 (22.0)	317	75 (23.7)	119	21 (17.6)	< 0.18 ³
PBC	436	6 (1.4)	317	5 (1.6)	119	1 (0.84)	< 0.99 ⁴
PSC	436	2 (0.46)	317	2 (0.63)	119	0 (0.0)	< 0.99 ⁴
24-hour urine output (cc)	400	1005.2 (454.8, 1589.8)	295	1090.0 (633.3, 1726.6)	105	563.8 (105.6, 1210.9)	< 0.001 ²
Variceal bleed	436	112 (25.7)	317	86 (27.1)	119	26 (21.8)	< 0.26 ³
Vasopressors on day of admission	436		317		119		< 0.001 ²
0		208 (47.7)		170 (53.6)		38 (31.9)	
1		175 (40.1)		126 (39.7)		49 (41.2)	
≥ 2		53 (12.12)		21 (6.63)		32 (26.9)	
Encephalopathy	436		317		119		< 0.43 ²
None		106 (24.3)		81 (25.6)		25 (21.0)	
Mild		123 (28.2)		88 (27.8)		35 (29.4)	
Moderate/severe		207 (47.5)		148 (46.7)		59 (49.6)	
Ascites	436		317		119		< 0.085 ²
None		114 (26.1)		86 (27.1)		28 (23.5)	
Mild		129 (29.6)		100 (31.5)		29 (24.4)	
Moderate/severe		193 (44.3)		131 (41.3)		62 (52.1)	
Labs and vitals							
Platelets (k/μL)	436	81.0 (56.5, 117.5)	317	83.0 (60.0, 117.0)	119	73.0 (49.0, 119.0)	< 0.21 ²
Prothrombin time (sec)	436	16.8 (14.2, 20.7)	317	15.6 (13.7, 18.0)	119	21.4 (18.3, 27.8)	< 0.001 ²
INR	436	1.5 (1.3, 1.9)	317	1.4 (1.2, 1.6)	119	2.0 (1.7, 2.6)	< 0.001 ²
Lactate (mmol/L)	341	2.3 (1.6, 3.4)	230	2.1 (1.4, 2.7)	111	3.0 (2.1, 5.4)	< 0.001 ²
MAP (mmHg)	436	65.0 (56.0, 106.0)	317	68.0 (60.0, 109.0)	119	58.0 (50.0, 68.0)	< 0.001 ²
ABG-pH	263	7.4 ± 0.10	170	7.4 ± 0.08	93	7.3 ± 0.12	< 0.001 ¹
ABG-PaCO ₂ (mmHg)	263	31.0 (27.0, 38.0)	170	31.0 (26.0, 37.0)	93	33.0 (27.0, 40.0)	< 0.098 ²
ABG-PaO ₂ (mmHg)	263	104.0 (80.0, 139.0)	170	113.0 (85.0, 147.0)	93	94.0 (76.0, 132.0)	< 0.019 ²

ABG-FiO ₂ (%)	263	40.0 (27.0, 55.0)	170	40.0 (25.0, 50.0)	93	44.0 (30.0, 70.0)	< 0.008 ²
PaO ₂ /FIO ₂ ratio	263	309.5 (195.0, 390.5)	170	337.5 (226.0, 426.7)	93	252.5 (143.0, 347.4)	< 0.001 ²
PAO ₂ (mmHg)	263	240.2 (151.0, 346.2)	171	231.5 (143.3, 324.0)	93	264.0 (168.4, 471.6)	< 0.008 ²
A-a gradient (mmHg)	263	119.8 (47.4, 233.9)	171	100.2 (38.6, 204.3)	93	166.7 (59.5, 319.6)	< 0.001 ²
Temperature (°C)	436	36.5 (36.2, 36.8)	317	36.5 (36.3, 36.8)	119	36.3 (35.4, 36.6)	< 0.001 ²
GCS	436	13.0 (8.0,14.0)	317	13.0(9.0,15.0)	119	11.0 (7.0, 14.0)	< 0.001 ²
Respiratory rate (rpm)	436	33.0 (26.0, 40.0)	317	33.0 (25.0, 39.0)	119	36.0 (27.0, 43.0)	< 0.052 ²
Heart rate (bpm)	436	92.8 ± 28.1	317	91.8 ± 25.7	119	95.5 ± 33.6	< 0.21 ¹
Hematocrit (%)	436	26.6 ± 5.9	317	27.1 ± 5.7	119	25.3 ± 6.2	< 0.005 ¹
WBC (k/μL)	436	7.5 (5.0, 11.4)	317	6.6 (4.6, 9.8)	119	10.7 (6.6, 17.7)	< 0.001 ²
Potassium (mmol/L)	436	4.0 (3.6, 4.8)	317	3.9 (3.5, 4.6)	119	4.4 (3.8, 5.1)	< 0.001 ²
BUN (mg/dL)	436	35.0 (21.0, 55.5)	317	32.0 (20.0, 51.0)	119	46.0 (28.0, 66.0)	< 0.001 ²
Albumin (g/dL)	436	2.7 (2.2, 3.1)	317	2.7 (2.3, 3.1)	119	2.7 (2.2, 3.3)	< 0.57 ²
Sodium (mmol/L)	436	136.6 ± 6.8	317	136.6 ± 6.6	119	136.4 ± 7.3	< 0.81 ¹
Creatinine (mg/dL)	436	1.6 (0.89, 2.8)	317	1.4 (0.80, 2.4)	119	2.2 (1.4, 3.5)	< 0.001 ²
Bilirubin (mg/dL)	435	4.2 (2.0, 10.4)	316	3.3 (1.7, 6.2)	119	11.7 (5.6, 25.9)	< 0.001 ²
Bicarbonate (mmol/L)	424	19.3 ± 5.4	310	19.9 ± 4.9	114	17.7 ± 6.3	< 0.001 ¹
Glucose (mg/dL)	430	152.6 ± 90.3	314	153.9 ± 94.0	116	149.1 ± 79.8	< 0.63 ¹
Scores							
MELD score	435	23.2 ± 9.8	316	20.3 ± 8.3	119	31.1 ± 8.9	< 0.001 ¹
MELD-Na score	435	24.8 ± 9.2	316	22.1 ± 8.1	119	32.0 ± 8.1	< 0.001 ¹
Child-Pugh score	435	10.8 ± 2.1	316	10.3 ± 2.1	119	11.9 ± 1.8	< 0.001 ¹
SOFA score	263	10.2 ± 3.5	170	9.0 ± 2.9	93	12.4 ± 3.4	< 0.001 ¹
CLIF-SOFA score	262	11.2 ± 3.5	169	9.9 ± 3.0	93	13.5 ± 3.2	< 0.001 ¹
RFH score	259	0.05 (-0.77, 1.1)	167	-0.30 (-0.99, 0.59)	92	0.96 (0.08, 2.1)	< 0.001 ²
APS	435	65.6 ± 28.4	317	58.0 ± 22.1	118	86.2 ± 32.8	< 0.001 ¹
APACHE III score	435	85.1 ± 28.3	317	77.7 ± 23.1	118	105.0 ± 31.5	< 0.001 ¹

Values presented as Mean ± SD, Median (P25, P75) or *n* (column %). *P* values:

¹ANOVA.

²Kruskal-Wallis test.

³Pearson's χ^2 test.

⁴Fisher's Exact test. ICU: Intensive care unit; COPD: Chronic obstructive pulmonary disease; A1AT: Alpha 1 anti-trypsin deficiency; AIH: Autoimmune hepatitis; ALD: Alcoholic liver disease; HCV: Hepatitis C virus; HBV: Hepatitis B virus; NASH: Non-alcoholic steatohepatitis; PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis; INR: International normalized ratio; MAP: Mean arterial pressure; ABG: Arterial blood gas; PaCO₂: Partial arterial pressure of carbon dioxide; PaO₂: Oxygen; FiO₂: Inspired oxygen concentration; A-a gradient: Alveolar arterial partial pressure oxygen gradient; FiO₂/PaO₂: Oxygenation index; GCS: Glasgow coma scale; rpm: Respirations per minute; bpm: Beats per minute; WBC: White blood cell count; BUN: Blood urea nitrogen; MELD: Model for end-stage liver disease; Na: Sodium; CPS: Child-Pugh score; SOFA: Sequential (or sepsis-related) organ failure assessment; CLIF-SOFA: Chronic liver failure-sequential organ failure assessment; RFH: Royal free hospital; APS: Acute physiology score; APACHE: Acute physiology and chronic health evaluation.

In multivariate analysis, a combination of the natural logarithm (ln) of the bilirubin, PT, WBC, and MAP was found to most accurately predict in-hospital mortality. Based on the regression coefficients of the independent variables (Table 2), a novel model to predict inpatient mortality was established. The final proposed model was defined as: $z = 1.19330 + [0.6137 \times \ln(\text{bilirubin})] - (47.203/PT) + (0.0715 \times WBC) - (0.0198 \times MAP)$. The *z* value is subsequently converted into a risk score to calculate probability of mortality utilizing the formula: $LIV-4 = \text{Probability of death (\%)} = [ez/(1 + ez)] \times 100$. Percentage values range from 0 to 100.

The Hosmer-Lemeshow goodness-of-fit X2 test was 5.4 (*P* = 0.72) and the AUROC for this model was 0.86 (95% CI: 0.82-0.90). Using bootstrap resampling, internal validation of the model was undertaken and produced an AUROC of 0.85. Based on Youden's index^[31] and using a cutoff of 26.5, the new score performed with a sensitivity of 81%, specificity of 76%, Positive Predictive Value of 58%, and Negative Predictive Value of 92%. Alternatively, a cutoff of 45.8 yields a sensitivity of 61% and specificity of 90%.

Comparison of prognostic models

Several scores demonstrated excellent accuracy for prediction of in-hospital mortality. The CLIF-SOFA and MELD scores, both liver-specific models, performed the best in the cohort with AUROCs of 0.81. The RFH score performed with an AUROC of 0.77. By comparison, ICU-specific scores, including the SOFA, APS and APACHE III

Table 2 Training cohort: Factors associated with in-hospital mortality: multivariate logistic regression with variable transformations

Factor	Estimate (95%CI)	OR (95%CI)	P value
Ln (Bilirubin)	0.61 (0.34, 0.89)	1.8 (1.4, 2.4)	< 0.001
1/PT	-47.2 (-67.3, -27.1)	0.09 (0.03, 0.26) ¹	< 0.001
WBC	0.07 (0.03, 0.11)	1.07 (1.03, 1.1)	< 0.001
MAP	-0.02 (-0.03, -0.01)	0.91 (0.86, 0.96) ²	< 0.001

¹OR corresponds to 0.05 increment in 1/PT.²OR corresponds to 5-unit increment in MAP. Ln: Natural logarithm; OR: Odds ratio; CI: Confidence interval; PT: Prothrombin time; WBC: White blood cell count; MAP: Mean arterial pressure.

performed with AUROCs of 0.79, 0.76, and 0.76, respectively. The liver intensive care unit variable-4 score (the LIV-4 score) performed higher than all other models, with an AUROC of 0.86. **Figure 1** displays AUROCs of the top-performing scores. DeLong *et al*^[30] method was employed to compare the predictive ability of the new model to that of the other scores. The LIV-4 score performed significantly better than the MELD, MELD-Na, Child-Pugh Score, RFH and APACHE III scores (**Table 3**).

Validation cohort

Table 4 presents a comparison of the training and validation cohort characteristics. A total of 336 cirrhotic patients were admitted between 2015-2017, of whom 107 (31.8%) died. Patients in the internal validation cohort were substantially sicker, as evidenced by higher MELD, MELD-Na, APACHE III, SOFA and LIV-4 scores. Despite differences between the cohorts, the LIV-4 score remained significantly higher in subjects who expired during the hospital stay (**Figure 2**) and exhibited good prognostic values in the validation cohort with an AUROC of 0.80 (**Figure 3**). There was no statistically significant difference between the LIV-4 score's AUROC from the training cohort and the validation cohort ($P = 0.11$). In the validation cohort, the SOFA score performed with an AUROC of 0.78, the APACHE III with an AUROC of 0.74, the MELD score with an AUROC of 0.80, the MELD-Na with an AUROC of 0.79, the CLIF-SOFA with an AUROC of 0.83, and the RFH with an AUROC of 0.64. The LIV-4 model performed with a significantly higher AUROC than the RFH [AUROC: 0.64 (0.56, 0.72)], and was non-inferior to other ICU- and liver-specific scores (**Table 5**). Using a cutoff of 26.5, LIV-4 continued to perform with a high negative predictive value of 89.1 (84.6, 93.6) (**Table 6**).

DISCUSSION

Our new model, the LIV-4 score, is calculated based on objective variables typically available at the time of ICU admission in patients with liver disease: The MAP, WBC, bilirubin, and PT. This combination of variables reflects hepatic and extra-hepatic (circulatory and immune) dysfunction, which are validated risk factors for mortality in patients with cirrhosis^[32-34]. This score performed better in our training cohort as a predictor for short-term mortality than other ICU- and liver-specific models, including the SOFA, CLIF-SOFA, and RFH scores, with excellent discriminative ability and calibration. In our validation cohort, it performed better than the RFH and was non-inferior to all others. In addition, the LIV-4 provides a survival probability score. This survival probability calculation may be useful for critical care, hepatology and surgical specialists when addressing goals and expectations of critical care with patients and their families. The APACHE methodology, APS, and SOFA were developed to assess the clinical course and predict survival of all-comers admitted to the ICU^[9-11]. Liver-specific scores, such as the Child-Pugh Score, MELD, MELD-Na and CLIF-SOFA are used to grade severity of liver disease, predict 90-day mortality, allocate organs for transplantation, and define acute-on-chronic liver failure^[1,12-15]. Liver-specific scores have been extrapolated for use as predictive models for mortality in the ICU, but have not performed better than ICU-specific scores^[17-25]. In our study, the MELD and the CLIF-SOFA scores (both liver-specific scores and both with AUROCs of 0.81), performed better than ICU-specific scores, including the SOFA, APACHE III, and APS scores (AUROCs of 0.79, 0.76, 0.76, respectively). We postulate that the differences in our observations relate to critical care trends over time, with associated improved survival and lower event-deaths in more recent years. Our model was formulated in a more contemporary cohort than previous models and was

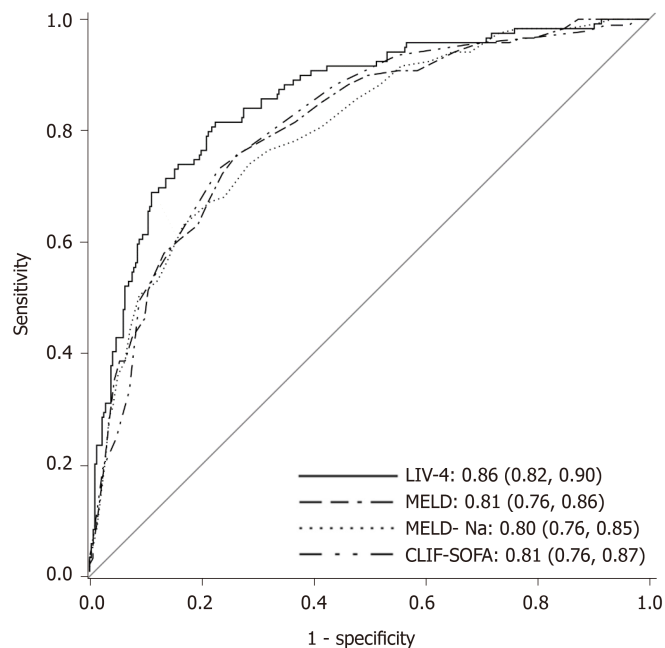


Figure 1 Training cohort: Predictive scores for in-hospital mortality in cirrhotic patients. LIV-4: LIV-4 score; MELD: Model for end-stage liver disease; MELD-Na: Model for end-stage liver disease-Sodium; CLIF-SOFA: Chronic liver failure-sequential organ failure assessment.

subsequently prospectively validated, with objective variables that more accurately reflect current critical care challenges in the approach to the cirrhotic patient—most notably circulatory/ adrenocortical dysfunction and infection/inflammation^[32-35]. Variable mortality trends with time were also observed in the development of the updated RFH score^[19]. Our mortality rates of 27.3% between 2008-2014 and 31.8% between 2015-2017 are similar to that of a comparable cohort from the Royal Free Hospital (2009-2012; 35.4%)^[19], as well as the cohort of patients described in the development of the CLIF-SOFA score (2011; 29.7% in patients with acute-on-chronic liver failure)^[1].

The RFH score is a liver-specific ICU score that has been previously externally validated in several centers in Scotland^[26]. Our study is the first in the United States to validate the updated RFH score, which performed in our cohort with an AUROC of 0.77. However, we found that the RFH score was limited in its generalizability as lactate and A-a gradient were not universally available on admission in our cohort. Lactate has been shown to be an independent predictor of mortality in cirrhotic patients^[18,19,26,27,36,37] and in patients with acute liver failure^[38] admitted to the ICU. However, lactate clearance has also been shown to be impaired by liver and extra-hepatic organ dysfunction, as evidenced by decreased clearance with increasing L-SOFA score^[39], which suggests that lactate levels may not be reliable in cirrhotic patients. Similarly, arterial blood gas analysis and calculation of the A-a gradient is more likely to be collected in patients with respiratory failure necessitating mechanical ventilation, and is not universally available in patients admitted to the ICU as the precise FiO₂ is often unknown. Finally, variceal hemorrhage as a reason for admission to the ICU was not an independent predictor of in-hospital mortality in our cohort. For these reasons, in an effort to create a widely applicable score for all cirrhotic patients admitted to the ICU, we did not include lactate, arterial blood gas analysis, or variceal hemorrhage in our new prognostic model. The LIV-4 performs with better discrimination and calibration in all patients with cirrhosis admitted to our ICU, independent of variceal hemorrhage, presence/grade of encephalopathy, and presence/degree of ascites.

In terms of limitations, patients were identified from the prospectively developed ICU APACHE IVb database and data was collected retrospectively. It is possible that all consecutive patients with cirrhosis were not captured with our retrospective methodology as a consequence of coding error, or if cirrhosis was not recognized as a pre-existing chronic health condition on admission to ICU. While internal prospective validation at our center suggests that the LIV-4 score will be widely applicable, we advocate for external, prospective analyses to be undertaken across diverse ICU settings in an effort to validate the clinical applicability of the score. Finally, it is

Table 3 Training cohort: Predictive abilities of critical care and liver-specific scores compared to the LIV-4 score

Score compared to LIV-4	P value
MELD	0.009
MELD-Na	0.002
Child-Pugh Score	< 0.001
SOFA	0.061
CLIF-SOFA	0.091
RFH	0.04
APS	0.001
APACHE III	0.002

MELD: Model for end-stage liver disease; Na: Sodium; SOFA: Sequential (or Sepsis-Related) organ failure assessment; CLIF-SOFA: Chronic liver failure-sequential organ failure assessment; RFH: Royal free hospital; APS: Simplified acute physiology score; APACHE: Acute physiology and chronic health evaluation.

important to recognize that, much as the APACHE scoring system has evolved to reflect progressive trends in the practice of critical care medicine, temporal study for re-calibration of LIV-4 will be necessary.

Patients with cirrhosis admitted to the ICU present unique clinical challenges for the clinician, and are best managed by a multidisciplinary team, comprised of specialists in both critical care and hepatology^[8]. Prognostication of short-term survival is important in order to identify patients at highest risk for mortality in terms of allocation of resources, studies and interventions. We report the development and prospective validation of a new prognostic model for the prediction of inpatient transplant-free survival in a contemporary cohort of cirrhotic patients admitted to the ICU. This tool can be easily accessed online at <http://riskcalc.org:3838/LIV-4/>. If external validation is undertaken, the LIV-4 score could become a standard clinical tool in the ICU and maybe used as a means of stratifying critically ill patients with cirrhosis in clinical and translational research studies.

Table 4 Validation cohort characteristics

Factor	Training cohort (n = 436)		Validation cohort A (n = 336)		P value
	n	Statistics	n	Statistics	
Gender	436		336		< 0.030 ²
Female		151 (34.6)		142 (42.3)	
Male		285 (65.4)		194 (57.7)	
Serum bilirubin	435	4.2 (2.0, 10.4)	336	5.0 (2.0, 13.5)	< 0.26 ¹
PT	436	16.8 (14.2, 20.7)	336	18.0 (14.8, 23.5)	< 0.003 ¹
WBC	436	7.5 (5.0, 11.4)	336	8.3 (5.0, 14.0)	< 0.040 ¹
MAP	436	65.0 (56.0, 106.0)	336	64.0 (56.0, 75.0)	< 0.26 ¹
Non-liver specific scores					
APACHE III	435	83.0 (65.0, 102.0)	333	88.0 (70.0, 109.0)	< 0.006 ¹
SOFA	263	10.0 (8.0, 12.0)	186	11.0 (8.0, 14.0)	< 0.003 ¹
Liver specific scores					
MELD	435	22.0 (16.0, 30.0)	336	25.0 (17.0, 33.0)	< 0.004 ¹
MELD-Na	435	24.0 (18.0, 31.0)	336	27.0 (20.0, 34.0)	< 0.006 ¹
CLIF-SOFA	262	11.0 (9.0, 13.0)	336	11.0 (9.0, 13.0)	< 0.53 ¹
RFH	259	0.05 (-0.77, 1.1)	184	0.83 (-0.18, 2.1)	< 0.001 ¹
LIV-4	435	16.8 (5.7, 43.6)	336	23.0 (7.1, 57.7)	< 0.007 ¹
Admission outcomes					
ICU LOS (d)	436	2.6 (1.4, 5.2)	336	3.7 (2.0, 7.6)	< 0.001 ¹
Hospital LOS (d)	436	8.7 (4.7, 16.8)	336	11.7 (5.7, 22.0)	< 0.002 ¹
Hospital discharge status	436		336		< 0.17 ²
Discharged alive		317 (72.7)		229 (68.2)	
In-hospital death		119 (27.3)		107 (31.8)	

¹Kruskal-Wallis test.²Pearson's χ^2 test. PT: Prothrombin time; WBC: White blood cell count; MAP: Mean Arterial Pressure; APACHE: Acute physiology and chronic health evaluation; SOFA: Sequential (or Sepsis-related) organ failure assessment; MELD: Model for end-stage liver disease; Na: Sodium; CLIF-SOFA: Chronic liver failure-sequential organ failure assessment; RFH: Royal Free Hospital; LOS: Length of stay.**Table 5 Validation cohort: Comparison of the various scores and LIV-4**

Score comparison	Validation cohort P value
MELD vs LIV-4	< 0.75
MELD-Na vs LIV-4	< 0.47
SOFA vs LIV-4	< 0.94
CLIF-SOFA vs LIV-4	< 0.27
RFH vs LIV-4	< 0.001
APACHE III vs LIV-4	< 0.074

Areas under the ROC curves were compared using De-Long's method. MELD: Model for end-stage liver disease; Na: Sodium; SOFA: Sequential (or Sepsis-related) organ failure assessment; CLIF-SOFA: Chronic liver failure-sequential organ failure assessment; RFH: Royal Free Hospital; APACHE: Acute physiology and chronic health evaluation; ROC: Receiver operator characteristics.

Table 6 Validity measures for LIV-4

Cohort	Measure	LIV-4 ≥ 26.5	LIV-4 ≥ 45.8
Validation Cohort	Sensitivity	81.3 (73.9, 88.7)	61.7 (52.5, 70.9)
	Specificity	71.2 (65.3, 77.0)	83.4 (78.6, 88.2)
	PPV	56.9 (49.0, 64.7)	63.5 (54.2, 72.7)
	NPV	89.1 (84.6, 93.6)	82.3 (77.4, 87.2)

Values presented as estimate (95%CI). PPV: Positive predictive value; NPV: Negative predictive value; CI: Confidence Interval.

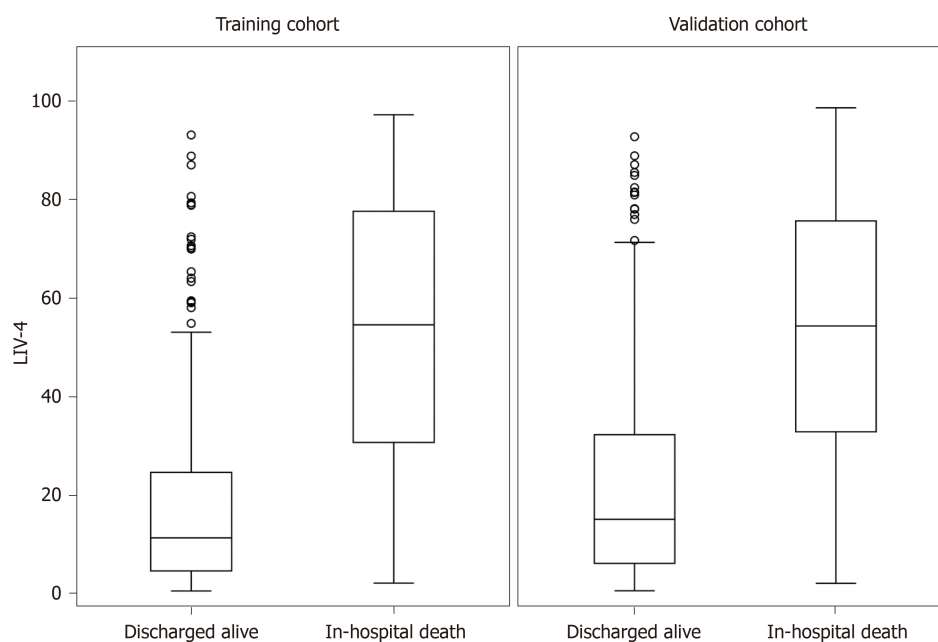


Figure 2 LIV-4 score is higher in subjects who expired during the hospital admission. The box-and-whisker plot is represented by the lower boundary of the box indicating the 25th percentile, the line within the box indicating the median value, the upper boundary of the box indicating the 75th percentile. The whiskers extend to the most extreme data point, which is no more than 1.5 times the interquartile range from the box, and the circles are outliers (values > 1.5 interquartile range).

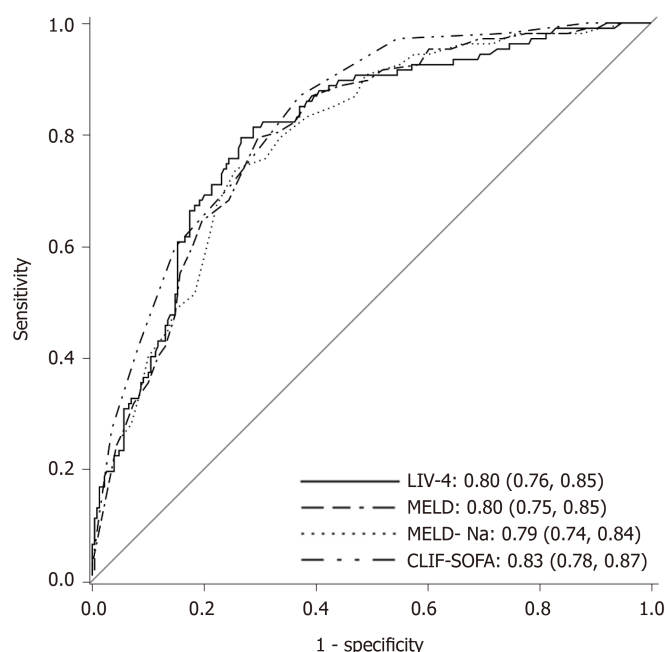


Figure 3 Validation cohort: Predictive scores for in-hospital mortality in cirrhotic patients. LIV-4: LIV-4 score; MELD: Model for end-stage liver disease; MELD-Na: Model for end-stage liver disease-Sodium; CLIF-SOFA: Chronic liver failure-sequential organ failure assessment.

ARTICLE HIGHLIGHTS

Research background

Critically ill patients with cirrhosis have higher mortality rates in the intensive care unit (ICU) than patients without chronic liver disease. Prognostication of short-term mortality is important in order to identify patients at highest risk of death. None of the currently available prognostic models have been widely accepted for use in cirrhotic patients in the ICU, perhaps due to complexity of calculation, or lack of universal variables readily available for these patients.

Research motivation

We believe a simple and widely applicable survival model can be developed, to guide therapeutic decision-making and contribute to cost-effective healthcare resource utilization.

Research objectives

To identify clinical and laboratory markers universally available at the time of ICU admission that best identify the likelihood of transplant-free survival in critically ill patients with cirrhosis.

Research methods

A new predictive model (the LIV-4 score) was developed retrospectively using logistic regression analysis from a large cohort of critically ill patients with cirrhosis admitted to a quaternary care liver transplant center ICU and was prospectively validated in a cohort of patients admitted to the same institution.

Research results

Our validated model for predicting mortality in cirrhotic patients on admission to the ICU performs better than previously published liver and ICU-specific scores.

Research conclusions

LIV-4 could become a standard clinical tool for patients with advanced liver disease in the ICU and could be used as a means of stratifying critically ill cirrhotic patients in clinical research studies.

Research perspectives

Survival modeling is an important tool for therapeutic decision-making as well as for research study design. The LIV-4 score was designed and validated prospectively in a single-center cohort. External, prospective validation is needed to determine widespread applicability and utility of the model.

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Observational Study

Low phospholipid-associated cholelithiasis syndrome: A rare cause of acute pancreatitis that should not be neglected

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Abstract

BACKGROUND

Low phospholipid-associated cholelithiasis (LPAC) syndrome is a very particular form of biliary lithiasis with no excess of cholesterol secretion into bile, but a decrease in phosphatidylcholine secretion, which is responsible for stones forming not only in the gallbladder, but also in the liver. LPAC syndrome may be underreported due to a lack of testing resulting from insufficient awareness among clinicians.

AIM

To describe the clinical and radiological characteristics of patients with LPAC syndrome to better identify and diagnose the disease.

METHODS

We prospectively evaluated all patients aged over 18 years old who were consulted or hospitalized in two hospitals in Paris, France (Bichat University Hospital and Croix-Saint-Simon Hospital) between January 1, 2017 and August 31, 2018. All patients whose profiles led to a clinical suspicion of LPAC syndrome underwent a liver ultrasound examination performed by an experienced radiologist to confirm the diagnosis of LPAC syndrome. Twenty-four patients were selected. Data about the patients' general characteristics, their medical history, their symptoms, and their blood tests results were collected during both their initial hospitalization and follow-up. Cytolysis and cholestasis were expressed compared to the normal values (N) of serum aspartate and alanine transaminase activities, and to the normal value of alkaline phosphatase level, respectively. The subjects were systematically reevaluated and asked about their symptoms 6 mo after inclusion in the study through an in-person medical appointment or phone call. Genetic testing was not performed systematically, but

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RESULTS

Most patients were young (median age of 37 years), male (58%), and not overweight (median body mass index was 24). Many had a personal history of acute pancreatitis (54%) or cholecystectomy (42%), and a family history of gallstones in first-degree relatives (30%). LPAC syndrome was identified primarily in patients with recurring biliary pain (88%) or after a new episode of acute pancreatitis (38%). When present, cytolytic and cholestasis were not severe (2.8N and 1.7N, respectively) and disappeared quickly. Interestingly, four patients from the same family were diagnosed with LPAC syndrome. At ultrasound examination, the most frequent findings in intrahepatic bile ducts were comet-tail artifacts (96%), microlithiasis (83%), and acoustic shadows (71%). Computed tomography scans and magnetic resonance imaging were performed on 15 and three patients, respectively, but microlithiasis was not detected. Complications of LPAC syndrome required hospitalizing 18 patients (75%) in a conventional care unit for a mean duration of 6.8 d. None of them died. Treatment with ursodeoxycholic acid (UDCA) was effective and well-tolerated in almost all patients (94%) with a rapid onset of action (3.4 wk). Twelve patients' (67%) adherence to UDCA treatment was considered "good." Five patients (36%) underwent cholecystectomy (three of them were treated both by UDCA and cholecystectomy). Despite UDCA efficacy, biliary pain recurred in five patients (28%), three of whom adhered well to treatment guidelines.

CONCLUSION

LPAC syndrome is easy to diagnose and treat; therefore, it should no longer be overlooked. To increase its detection rate, all patients who experience recurrent biliary symptoms following an episode of acute pancreatitis should undergo an ultrasound examination performed by a radiologist with knowledge of the disease.

Key words: Low phospholipid-associated cholelithiasis syndrome; Acute pancreatitis; Cholelithiasis; Echography; Ursodeoxycholic acid

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Core tip: Low phospholipid-associated cholelithiasis (LPAC) syndrome is considered rare, but it may be underreported due to a lack of testing resulting from insufficient awareness among physicians, radiologists, and digestive surgeons. This study describes the clinical and radiological characteristics of patients with LPAC syndrome, which helps clinicians better diagnose and treat the disease. Diagnosis is easily made *via* ultrasound imaging performed on patients with typical recurring biliary symptoms, and medical treatment with ursodeoxycholic acid is rapidly effective and well-tolerated. LPAC syndrome is straightforward to diagnose and treat; therefore, it should no longer be overlooked.

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INTRODUCTION

Low phospholipid-associated cholelithiasis (LPAC) syndrome is a very particular form of biliary lithiasis that was first described by Rosmorduc *et al*^[1] in 2001. Contrary to what occurs in "common" cholelithiasis, there is no excess of cholesterol secretion into bile in this syndrome, but a decrease in phosphatidylcholine secretion. Phosphatidylcholine is the major phospholipid in human bile; *i.e.*, it forms micelles to solubilize cholesterol and permit its transportation. With a low phospholipid content,

the bile is oversaturated with cholesterol crystals that precipitate to form microscopic and macroscopic stones not only in the gallbladder but also in the liver^[2]. A decrease in phosphatidylcholine secretion is caused by a mutation of ATP-binding cassette, subfamily B, member 4 (*ABCB4*) gene encoding the bile canalicular protein Multidrug resistance 3 (MDR3), which is the phosphatidylcholine translocator across the canalicular membrane of the hepatocyte^[3].

The prevalence of LPAC syndrome has not been evaluated with precision, but it affects around 5% of patients with symptomatic cholelithiasis^[2]. Although only a few studies have described patients with this disorder due to its rarity and its recent discovery, several common characteristics have been identified. LPAC syndrome usually manifests through biliary symptoms or complications (*e.g.*, biliary pain, acute pancreatitis, cholecystitis, or cholangitis) that occur in young patients who are not overweight. These symptoms often recur after cholecystectomy. Moreover, patients frequently report a family history of cholelithiasis, or a personal history of intrahepatic cholestasis of pregnancy in women^[3].

Ultrasound examination of the liver is essential for diagnosis because it can reveal intrahepatic stones, which appear as heterogeneous and echoic foci with acoustic shadows centered on the intrahepatic ducts, intrahepatic microlithiasis, or comet-tail artifacts due to ultrasound reverberation^[4].

Although the reason behind its efficacy is not completely understood, treatment with ursodeoxycholic acid (UDCA) seems to diminish symptoms of LPAC syndrome in the majority of cases^[1,3,5]. Such treatment is typically carried out on a long-term basis, but it remains unclear whether the therapy should be administered throughout a patient's entire life. Improving the understanding of this syndrome will facilitate the screening and treatment of patients.

LPAC syndrome may be underreported due to a lack of testing resulting from insufficient awareness among physicians, radiologists, and digestive surgeons. The aim of this study was to describe the clinical and radiological characteristics of patients with LPAC syndrome to better identify and diagnose the disease.

MATERIALS AND METHODS

Patients

We prospectively evaluated all patients aged over 18 years old who were consulted or hospitalized in two hospitals in Paris, France (Bichat University Hospital and Croix-Saint-Simon Hospital) between January 1, 2017 and August 31, 2018. All patients whose profiles led to a clinical suspicion of LPAC syndrome underwent a liver ultrasound examination performed by an experienced radiologist to confirm the diagnosis of LPAC syndrome.

Patients were excluded from the study if they had an acute pancreatitis with any other etiology, if they suffered from chronic alcoholism or, if at the ultrasound examination, their liver was dysmorphic or had massive steatosis.

Informed consent was obtained from all subjects.

Data acquisition

Data about the patients' general characteristics, their medical history, their symptoms, and their blood tests results, were collected during both their initial hospitalization and follow-up. Cytolysis and cholestasis were expressed compared to the normal values (N) of serum aspartate and alanine transaminase activities, and to the normal value of alkaline phosphatase level, respectively.

LPAC syndrome was suspected when at least one of the following features was present: The onset of biliary pain before the age of 30 years; biliary pain recurring after cholecystectomy; a personal history of acute pancreatitis with unknown etiology; a personal history of pregnancy cholestasis; or a family history of gallstones before the age of 30 years in first-degree relatives.

The diagnosis was made by ultrasound examination when the following findings were detected in the intrahepatic bile ducts: Hyperechoic foci in the form of comet-tail artifacts, microlithiasis, or stones with acoustic shadows.

Radiological features during ultrasound examinations were interpreted and reported by a skilled radiologist who was familiar with LPAC syndrome. All ultrasound examinations were performed using an Aplio 500 ultrasound machine with a 3.5 MHz transducer (Toshiba, Tokyo, Japan).

Follow-up

The subjects were systematically reevaluated and asked about their symptoms 6 months after inclusion in the study through an in-person medical appointment or

phone call. Follow-up was based on clinical evaluation and not on ultrasound examination. The efficacy of UDCA treatment was defined as the complete disappearance or a significant decrease of biliary pain intensity or frequency. Intolerance to UDCA treatment was defined as the occurrence of side effects that required either stopping treatment or lowering dosage. Patients' adherence to UDCA treatment was considered "good" when taking the medication at least six days per week. Adherence was defined as "poor" when patients missed more than one day of medication per week, or if they stopped taking it without having been requested by their physician.

Genetic testing

Genetic testing was not performed systematically, but according to the decision of each physician, and written consent was obtained from all patients involved. EDTA whole-blood samples were sent to the genetic laboratory of Saint-Antoine Hospital in Paris. Molecular analysis was performed by sequencing the coding exons and the adjacent intron junctions of all the genes implicated in hereditary cholelithiasis. These genes were *ABCB4/MDR3* (NM_00043), *ABCB11/BSEP* (NM_003742), *ATP8B1/FIC1* (NM_005603), *ABCC2/MRP2* (NM_000392), *NR1H4/FXR* (NM_005123), *ABCG5/ABCG5* (NM_022436), *ABCG8/ABCG8* (NM_022437), *SLC4A2/AE2* (NM_003040), *GPBAR1/TGR5* (NM_001077191), and *AQP8* (NM_001169). DNA was amplified with primers specific for the coding exons and their intron boundaries. Sequencing was performed by capture (NimbleGen; Roche, Basel, Switzerland) and next-generation sequencing (MiSeq; Illumina, San Diego, CA, United States), and the results were analyzed with SOPHiA DMM software (SOPHiA Genetics, Boston, MA, United States). When gene variants were detected, results were confirmed on polymerase chain reaction products using the Sanger sequencing technique.

Statistical analysis

Quantitative variables were expressed as means \pm standard deviation (SD) and qualitative variables were expressed as *n* (%).

RESULTS

Patients' characteristics at diagnosis

Twenty-four patients prospectively seen in the two hospitals between January 1, 2017 and August 31, 2018 had both clinical and radiological signs of LPAC syndrome, met none of the exclusion criteria, and were thus included in the study. The characteristics of the 24 patients are presented in [Table 1](#).

Patients with LPAC syndrome were for the most part young with a median age of 37 years, mostly male (58%), and not overweight [median body mass index (BMI) was 24]. Many had a medical history of acute pancreatitis (54%) and cholecystectomy (42%). Interestingly, a family history of gallstones in first-degree relatives was frequent (30%). None of the female patients were pregnant, had a history of cholestasis of pregnancy, or were treated by estrogen therapy.

LPAC syndrome was identified primarily in patients who experienced recurring biliary pain (88%). Only three patients (12%) had no symptoms (they had an ultrasound examination due to a family history of LPAC syndrome). Acute pancreatitis was the most frequent complication that led to the identification of the disease, occurring in nine cases (38%) *vs* two cases (14%) of acute cholecystitis and only one case (4%) of acute cholangitis. Liver function was not altered; we observed cytolysis and cholestasis in 13 patients (57%). When present, cytolysis and cholestasis were not severe (2.8N and 1.7N, respectively) and disappeared quickly, as only four of 19 patients (21%) still had cytolysis and cholestasis when the ultrasound examinations were performed.

Genetic testing was performed on seven patients, none of which had a mutation in the *ABCB4/MDR3* gene.

Interestingly, four patients from the same family were diagnosed with LPAC syndrome. Their family tree is presented in [Figure 1](#).

Patient 1 was the father of Patients 2 and 3. He had a medical history of several episodes of acute pancreatitis, cholecystitis, and cholangitis, and underwent cholecystectomy. LPAC syndrome was diagnosed after the occurrence of a new episode of acute pancreatitis. Patients 2 and 3 had no significant medical history or symptoms and underwent ultrasound examinations because of their father's diagnosis. Patient 4 was the daughter of Patient 1's brother. She complained of recurring biliary pain and the diagnosis was made by echography. An ultrasound examination was not performed on Patient 4's father; therefore, we cannot know if he

Table 1 Patients' characteristics at diagnosis

General characteristics			
Median age (yr)	37 ± 11.8	(26–67)	<i>n</i> = 24
Gender: Male	14	(58)	<i>n</i> = 24
Median BMI (kg/m ²)	24 ± 4.5	(18.9–34)	<i>n</i> = 24
Pregnancy at diagnosis	0	(0)	<i>n</i> = 10
Ethnicity			<i>n</i> = 24
Caucasian	12	(50)	
Maghrebian	10	(42)	
Asian	1	(4)	
Sub-Saharan African	1	(4)	
Medical history			
Acute pancreatitis	13	(54)	<i>n</i> = 24
Cholecystectomy	10	(42)	<i>n</i> = 24
Family history of cholelithiasis in first-degree relatives	7	(30)	<i>n</i> = 23
Acute cholecystitis	3	(13)	<i>n</i> = 24
Acute cholangitis	3	(13)	<i>n</i> = 24
Chronic pancreatitis	0	(0)	<i>n</i> = 24
Cholestasis of pregnancy	0	(0)	<i>n</i> = 10
Estrogen therapy	0	(0)	<i>n</i> = 10
Existing medical conditions			
Recurring pain	21	(88)	<i>n</i> = 24
Acute pancreatitis	9	(38)	<i>n</i> = 24
Acute cholecystitis	2	(14)	<i>n</i> = 14
Acute cholangitis	1	(4)	<i>n</i> = 24
Cytolysis			<i>n</i> = 23
Presence	13	(57)	
Quantification (N)	2.8 ± 3.1	(0–10)	
Cholestasis			<i>n</i> = 23
Presence	13	(57)	
Quantification (N)	1.7 ± 2.0	(0–8)	

Quantitative results: means ± SD. Qualitative results: *n* (%); BMI: Body mass index; N: Normal value.

had LPAC syndrome.

Genetic testing was only performed on *Patient 1* who had a heterozygous missense variant c.634G>A, p.Ala212Thr in the *AQP8* gene. This mutation is not responsible for LPAC syndrome, but it can increase the risk of developing biliary lithiasis.

Radiological features

The diagnosis of LPAC syndrome was made by ultrasound examination performed by an experienced radiologist. The most frequent findings in intrahepatic bile ducts were comet-tail artifacts in 23 patients (96%), microlithiasis in 20 patients (83%), and acoustic shadows in 17 patients (71%). Stones were present only in three patients (13%). Some of the ultrasound findings are presented in [Figure 2](#).

Among patients who had no history of cholecystectomy, gallstones were detected in six (43%), and gallbladder sludge was found in two (14%). Other associated findings were less frequent. The detailed results of the ultrasound examinations are presented in [Table 2](#).

Computed tomography (CT) scans and magnetic resonance imaging (MRIs) were performed on 15 and three patients, respectively, but microlithiasis was not detected.

Outcome

The mean follow-up time was 19.7 mo. Complications of LPAC syndrome required hospitalizing 18 patients (75%) in a conventional care unit for a mean duration of 6.8 days. No patients needed to be hospitalized in an intensive care unit. Treatment with UDCA was administered to 18 patients (75%). Among the six patients who were not treated, three were asymptomatic and three refused to take the medication. Twelve patients' (67%) adherence to UDCA treatment was considered "good." Five patients

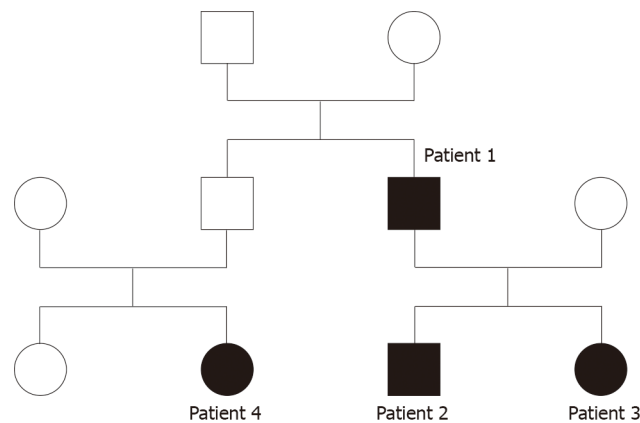


Figure 1 Family tree of four patients with low phospholipid-associated cholelithiasis syndrome. This family tree represents four patients with low phospholipid-associated cholelithiasis syndrome. Circles and squares indicate females and males, respectively. Clear symbols indicate unaffected individuals. Black symbols indicate affected individuals.

(36%) underwent cholecystectomy (three of them were treated both by UDCA and cholecystectomy). None of the 24 patients had to undergo a Roux-en-Y procedure. These results are presented in Table 3.

Treatment with UDCA was effective in 17 patients (94%). The onset of action was rapid (3.4 wk), and UDCA was well-tolerated: *e.g.*, only two patients (11%) experienced side effects (nausea and diarrhea) which required lowering their dosage. No patients experienced side effects strong enough to completely stop the treatment. Despite UDCA efficacy, biliary pain recurred in five patients (28%), three of whom adhered well to treatment guidelines. Interestingly, among these five patients, four had a genetic susceptibility that could contribute to the recurrence of symptoms. These genetic mutations were not identified as those responsible for LPAC syndrome but could be responsible for genetic cholelithiasis and pancreatitis. Each of the four patients had one of the following mutations: A heterozygous mutation in the gene *ATP8B1*, a homozygous missense mutation in the gene *ABCB11/BSEP*, a double heterozygous mutation $\Delta F508/L967S$ in the *CFTR* gene, and a heterozygous missense variant c.3220A>G, p.N1074D in the *CASR* gene.

No patients died.

DISCUSSION

Our original study prospectively evaluated 24 patients with LPAC syndrome. Our work determined not only the patients' characteristics, but also the symptoms and clinical signs that should lead physicians to search for the syndrome, the radiological features essential for diagnosis, and the treatment outcome.

Two limitations in our study should be highlighted: *i.e.*, the relatively limited number of patients included and the fact that genetic testing was not performed systematically, which hinders the analysis of all possible mutations present.

The prevalence of LPAC syndrome has not been described precisely in the literature, but it seems to be more frequent in our study than previously expected, with 24 patients diagnosed in less than 2 years at two medium-size hospitals. Contrary to "common" cholelithiasis, whose risk factors include being older, overweight, or female^[6], LPAC syndrome affects young patients with normal BMI. Even if most patients in our study were male (58%), the literature shows that the syndrome seems to occur more frequently among female patients, with a sex ratio close to 3:1^[1,3,5,7]. In a study conducted by Condat *et al*^[8], LPAC syndrome was responsible for 22% of cholelithiasis among women younger than 30 years old. In the same study, the average age of female patients was 24.7 years, which is younger than the average age observed among males (between 38 to 40 years in our study and in the literature)^[1,5]. Moreover, a previous study showed that the syndrome was associated with a history of intrahepatic cholestasis of pregnancy^[8]. These results could be explained by the aggravating role of estrogen that inhibits phospholipid excretion into bile^[9]. As LPAC syndrome is associated with particular genetic mutations, suspicion of the disease is strengthened in the case of a family history of cholelithiasis (evident in 30% of the patients in our study).

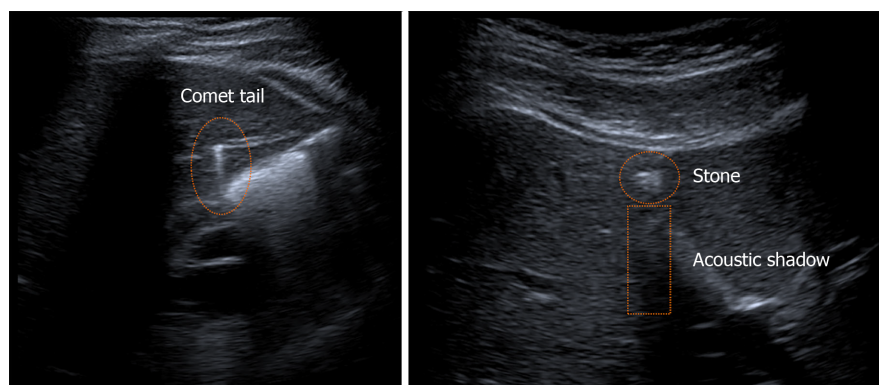


Figure 2 Ultrasound images of intrahepatic bile duct findings. Images courtesy of Dr. Karila-Cohen, Department of Radiology, AP-HP Bichat University Hospital, Paris 75018, France. This figure presents some of the intrahepatic bile duct findings detected via ultrasound imaging in our study. On the left: a comet-tail artifact. On the right: a stone and an acoustic shadow. These unpublished images are owned and were provided by Dr. Karila-Cohen.

Most patients are symptomatic with typical biliary pain leading to cholecystectomy in 90% of cases^[3]. The recurrence of symptomatology after cholecystectomy is due to intrahepatic lithiasis or lithiasis migration. Acute pancreatitis is a frequent complication of LPAC syndrome, as shown by the fact that nearly half of our patients had a personal history of acute pancreatitis or cholecystectomy, and more than one-third were diagnosed following a new episode of acute pancreatitis. Other complications such as acute cholecystitis and acute cholangitis are less frequent because most patients have already undergone cholecystectomy.

Ultrasound examination is of critical importance in the positive diagnosis of LPAC syndrome and must be carried out and interpreted by a radiologist familiar with the disease. The detection rate of signs of LPAC syndrome ranges from 5% if the radiologist is not familiar with the disease to 90% for an experienced radiologist^[10]. Comet-tail artifacts are the most common findings and must be distinguished from pneumobilia (contrary to pneumobilia, comet-tail artifacts are not mobile^[2]). CT scans are inefficient for diagnosis because their ability to detect microlithiasis is lower than that of echography. Biliary MRIs are usually normal^[10]. Other investigations such as echoendoscopy or liver biopsy are not relevant^[11] and an analysis of bile composition to detect low phospholipid concentration cannot be performed in clinical practice^[3].

LPAC syndrome is associated with mutations of the *ABCB4* gene located on chromosome 7, locus 21 (7q21), which codes for protein MDR3^[1,3]. Nevertheless, as diagnosis is based on clinical and radiological criteria, we chose not to systematically perform genetic testing in our study. Moreover, in the literature, mutations were detected in only 50%–65% of the patients suffering from LPAC syndrome^[2,3,7,12]. Hypotheses to explain the low mutation detection rate could be the presence of mutations in the introns, mutations in a gene promoter, mutations in a regulatory region, or mutations of another gene or biliary carrier (*ABCB11* or *BSEP*, *ABCC2*, *ABCG5/ABCG8*)^[2,3,7,13]. Nevertheless, genetic testing can be recommended in some situations. Searching for mutations in first-degree relatives can be done as genetic family counseling with a family screening. For research purposes, searching for mutations of the *ABCB4* gene should facilitate detection of new mechanisms responsible for the syndrome and render genetic screening more effective^[3,7,8,10].

The incidence of long-term complications, such as cirrhosis or malignant transformation, is still unknown, but sporadic cases have been described in the literature. Chronic aggression of the bile epithelium by lithiasis and hydrophobic bile acids can lead to the chronic inflammation responsible for secondary biliary cirrhosis^[3,7,14,15] and secondary sclerosis cholangitis^[4,16]. Moreover, dysplasia can ensue from chronic inflammation and cases of cholangiocarcinoma have been described among patients with LPAC syndrome^[2,7,12,16-18].

Medical treatment with UDCA is effective and has a rapid and positive impact on symptomatology (94% efficacy with an average onset of action of 3.4 weeks in our study). This observation suggests that symptoms are not directly related to stones, but may be due to inflammation of intrahepatic bile ducts or to cholesterol crystals not detected by echography^[2]. UDCA is usually well tolerated and side effects, such as diarrhea and nausea, are rare. Recommended daily dosage varies from 7 to 10 mg/kg, but can be increased to 20 mg/kg if biliary symptoms persist^[1,2,7]. UDCA is a long-term treatment that should be continued even if all symptoms have disappeared. There are no recommendations about dietary regimens, but in the case of associated

Table 2 Radiological features unveiled during ultrasound examinations

Intrahepatic bile duct findings			<i>n</i> = 24
Comet-tail artifacts	23	(96)	
Microlithiasis	20	(83)	
Acoustic shadows	17	(71)	
Stones	3	(13)	
Associated findings			
Gallstones	6	(43)	<i>n</i> = 14
Gallbladder sludge	2	(14)	<i>n</i> = 14
Gallbladder hydrops	1	(7)	<i>n</i> = 14
Common bile duct stones	1	(4)	<i>n</i> = 23

Qualitative results: *n* (%). Quantitative results: means \pm SD.

hypercholesterolemia, treatment with statin is preferable to fibrate because the latter increases bile lithogenicity^[2,3]. However, the appropriateness of surgical treatment is not clearly determined. In our study, we performed cholecystectomy in one-third of patients in addition to UDCA. The absence of guidelines about the role of cholecystectomy as a treatment for LPAC syndrome is mainly due to the difficulty of determining whether the symptomatology is ascribed to gallbladder lithiasis or to intrahepatic damage^[2,3]. Hence, one of the options is to reserve cholecystectomy in case of acute cholecystitis or if treatment with UDCA fails^[10]. If performed, cholecystectomy should always be done in addition to medical treatment with UDCA. If done without UDCA treatment, symptoms reoccur in half of patients^[1].

In conclusion, LPAC syndrome is likely underreported due to a lack of testing resulting from insufficient awareness among physicians, radiologists, and digestive surgeons. A deeper understanding of this disease by these medical professionals is necessary to avoid overlooking its rather simple diagnosis. LPAC syndrome typically manifests through biliary symptoms or episodes of acute pancreatitis in young patients with normal BMI. Symptoms often reoccur after cholecystectomy and patients usually have family history of cholelithiasis. The diagnosis is made *via* ultrasound examination by detecting intrahepatic lithiasis or comet-tail artifacts. Other exams are not necessary unless ultrasound examination proves ineffective. Genetic testing is not necessary for diagnosis because no mutations are detected in half of patients, but it can be performed for the purpose of research or genetic family counseling. LPAC syndrome is easily treatable with UDCA, which is rapidly effective, well-tolerated, and avoids the recurrence of biliary symptoms and long-term complications. Cholecystectomy should be reserved in the case of acute cholecystitis or if treatment with UDCA fails. LPAC syndrome is easy to diagnose and treat; therefore, it should no longer be overlooked. To increase its detection rate, all patients who experience recurrent biliary symptoms following an episode of acute pancreatitis should undergo an ultrasound examination performed by a radiologist with knowledge of the disease.

Table 3 Patients' characteristics during follow-up

Follow-up time (mo)	19.7 ± 5.8	(10.1–29.4)	n = 24
Patients' care			
Hospitalization			
In a conventional care unit			
Yes	18	(75)	n = 24
Duration (days)	6.8 ± 3.1	(2–14)	n = 18
In an intensive care unit			
	0	(0)	n = 24
Treatment			
UDCA	18	(75)	n = 24
Cholecystectomy	5	(36)	n = 14
Outcome			
Good adherence to UDCA	12	(67)	n = 18
UDCA efficacy	17	(94)	n = 18
Onset of action (in weeks)	3.4 ± 2.5	(2–12)	n = 17
Pain recurrence	5	(28)	n = 18
UDCA intolerance	2	(11)	n = 18
Death	0	(0)	n = 24

Qualitative results: n (%). Quantitative results: means ± SD. UDCA: Ursodeoxycholic acid.

ARTICLE HIGHLIGHTS

Research background

Low phospholipid-associated cholelithiasis (LPAC) syndrome is a very particular form of biliary lithiasis with no excess of cholesterol secretion into bile, but a decrease in phosphatidylcholine secretion, which is responsible for stones forming not only in the gallbladder, but also in the liver. This study describes the clinical and radiological characteristics of patients with LPAC syndrome to better identify and diagnose the disease.

Research motivation

LPAC syndrome is considered a rare disease, but it may be underreported due to a lack of testing resulting from insufficient awareness among physicians, radiologists, and digestive surgeons. Improving the understanding of this syndrome will facilitate the screening and treatment of patients.

Research objectives

We aimed to determine the clinical and radiological characteristics, as well as the outcome of patients with LPAC syndrome in order to better identify and diagnose the disease.

Research methods

We prospectively evaluated all adult patients who were consulted or hospitalized in two hospitals in Paris, France, between January 1, 2017 and August 31, 2018. All patients whose profiles led to a clinical suspicion of LPAC syndrome underwent a liver ultrasound examination performed by an experienced radiologist to confirm the diagnosis of LPAC syndrome. Twenty-four patients were selected. Patients' characteristics, radiological features and outcomes were analyzed.

Research results

Most patients were young (median age of 37 years), male (58%), and not overweight (median body mass index was 24). Many had a personal history of acute pancreatitis (54%) or cholecystectomy (42%), and a family history of gallstones in first-degree relatives (30%). LPAC syndrome was identified primarily in patients with recurring biliary pain (88%) or after a new episode of acute pancreatitis (38%). When present, cytotoxicity and cholestasis were not severe and disappeared quickly. Interestingly, four patients from the same family were diagnosed with LPAC syndrome. At ultrasound examination, the most frequent findings in intrahepatic bile ducts were comet-tail artifacts (96%), microlithiasis (83%), and acoustic shadows (71%). Computed tomography scans and magnetic resonance imaging were performed on 15 and three patients, respectively, but microlithiasis was not detected. Complications of LPAC syndrome required hospitalizing 18 patients (75%) in a conventional care unit for a mean duration of 6.8 d. None of them died. Treatment with ursodeoxycholic acid (UDCA) was effective and well-tolerated in almost all patients (94%) with a rapid onset of action (3.4 wk). Twelve patients' (67%) adherence to UDCA treatment was considered "good." Five patients (36%) underwent cholecystectomy (three of them were treated both by UDCA and cholecystectomy). Despite

UDCA efficacy, biliary pain recurred in five patients (28%), three of whom adhered well to treatment guidelines.

Research conclusions

LPAC syndrome is easy to diagnose and treat; therefore, it should no longer be overlooked. LPAC syndrome typically manifests through biliary symptoms or episodes of acute pancreatitis in young patients with normal BMI. Symptoms often reoccur after cholecystectomy and patients usually have family history of cholelithiasis. The diagnosis is made *via* ultrasound examination by detecting intrahepatic lithiasis or comet-tail artifacts. Other exams are not necessary unless ultrasound examination proves ineffective. Genetic testing is not necessary for diagnosis because no mutations are detected in half of patients, but it can be performed for the purpose of research or genetic family counseling. LPAC syndrome is easily treatable with UDCA, which is rapidly effective, well-tolerated, and avoids the recurrence of biliary symptoms and long-term complications. Cholecystectomy should be reserved in the case of acute cholecystitis or if treatment with UDCA fails.

Research perspectives

LPAC syndrome is likely underreported due to a lack of testing resulting from insufficient awareness among physicians, radiologists, and digestive surgeons. A deeper understanding of this disease by these medical professionals is necessary to avoid overlooking its rather simple diagnosis. To increase its detection rate, all patients who experience recurrent biliary symptoms following an episode of acute pancreatitis should undergo an ultrasound examination performed by a radiologist with knowledge of the disease.

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Prospective Study

Non-alcoholic fatty liver disease is not independent risk factor for cardiovascular disease event: A cohort study

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Abstract**BACKGROUND**

There are no consistent results between previous studies for an independent association between non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD) events.

AIM

To determine if there is an independent association between NAFLD and CVD events.

METHODS

In the present study, valid outcome data of 4808 subjects were available for phase 2 of our cohort study. These subjects had been followed up for seven years from phase 1, beginning in 2009-2010 to phase 2 during 2016-2017. Simple and multiple

Institutional review board

statement: This project was approved by the ethics committee of Iran University of Medical Sciences, Tehran, Iran by No IR.IUMS.REC.1397.1232.

Informed consent statement: An informed consent was obtained from all patients in both phases and recorded in their health documents before interviews and paraclinical tests.

Conflict-of-interest statement:

There is not any potential conflict of interest that might constitute an embarrassment to any of the authors.

Data sharing statement: There is no additional data available.

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Cox proportional models were used to determine the association between NAFLD in the primary phase of the cohort and subsequent fatal and non-fatal CVD events during follow-up.

RESULTS

The incidence of non-fatal CVD events in males with NAFLD was significantly higher ($P = 0.004$) than in males without NAFLD. A positive association was demonstrated between NAFLD and non-fatal CVD events in males (Hazard ratio = 1.606; 95%CI: 1.166-2.212; $P = 0.004$) by the simple Cox proportional hazard model, but no independent association was detected between these in the multiple Cox models.

CONCLUSION

No independent association was detected between NAFLD and CVD. It is likely that diabetes mellitus and age may be the principle mediators in this regard.

Key words: Non-alcoholic fatty liver disease; Cardiovascular disease; Cohort; Type 2 diabetes mellitus; Risk factor; Follow up

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Core tip: We evaluated the association between non-alcoholic fatty liver disease and the incidence of cardiovascular disease events after seven years follow up, in a large prospective cohort study. Based on our results, no independent association was observed between non-alcoholic fatty liver disease and cardiovascular disease events. Diabetes and age may play a role as potential mediators.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease worldwide^[1]. It is defined as the presence of hepatic steatosis after excluding other causes of hepatic fat accumulation such as excessive alcohol consumption, viruses and drug-related hepatitis^[2]. The global prevalence of NAFLD was estimated to be 25.2% in a meta-analysis of 86 studies by Younossi *et al*^[3]. The increase in the prevalence of NAFLD has usually followed the obesity pandemic in children and adults globally although a considerable fraction of subjects are lean. In particular, childhood obesity has implications for a greater risk of NAFLD later with accompanying liver-related diseases at a much lower age threshold^[1].

A number of hepatic complications from simple steato-hepatitis to cirrhosis and hepatocellular carcinoma can be attributed to NAFLD^[4]. This disease is considered to be a hepatic manifestation of metabolic syndrome^[5]. Thus, in addition to the relationship with liver related diseases and its complications, this condition may be associated with metabolic co-morbidities such as diabetes mellitus (DM) and dyslipidemia^[6-12]. The non-liver related deaths remain far more common than liver-related deaths^[3].

We previously showed that there is a significant association between NAFLD and 10-year cardiovascular disease (CVD) risk as estimated by well known risk assessment tools^[13]. These tools estimate CVD risk based on a cluster of established modifiable and non-modifiable risk factors for the development of CVD. Although previous studies have shown a positive association between CVD events and NAFLD, results showing an independent association between them are inconsistent^[14-16]. This study was conducted to determine an independent association, if any, between NAFLD and CVD events.

MATERIALS AND METHODS

Study setting and sampling frame

A comprehensive data collection was undertaken in two phases in our cohort study in Amol. Amol, a relatively well-populated city in the central area of northern Iran, is located 180 km from the city of Tehran. Phase 1 began in 2009-2010 and phase 2 in 2016-2017. The exact sampling frame was obtained using primary health records of subjects from local primary health care centers in urban and rural areas. The sampling frame, based on the primary data, comprised 16 strata according to gender and the following age group ranges within 10-90 years: 10-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79 and 80-89. From phase 1 to the beginning of phase 2 in 2016, participants or close family members were contacted annually to provide the outcomes related to fatal and non-fatal CVD. The comprehensive evaluations of the demographic, anthropometric and laboratory variables began in early phase 2 and these participants were actively invited to participate in this phase of the cohort study. Valid data, following exclusions (Figure 1) was obtained, from 4808 participants, in the present study. Figure 1 shows the study population and related inclusions and exclusions.

Outcomes

Atherosclerosis CVD (ASCVD) is defined based on the history of non-fatal acute myocardial infarction, ischemic heart disease death and cerebrovascular accidents. The incidence of ASCVD and the number of occurrences was considered as outcomes. Comprehensive assessment in phase 2 on the related outcomes was undertaken after data collection from self-reporting of patients, or close family members and by direct observation of valid medical records. Hospital discharge records and death certificates for fatal CVD events were evaluated. Active communications with the locations in which the patients were hospitalized, were pursued to establish medical record accuracy. In all instances, the related outcomes provided annually by the patient or a close family member were compared with data from valid documents, and modified accordingly in the case of inconsistent findings. Confirmation of associated outcomes was undertaken by an internist in the cohort study group.

The diagnosis of NAFLD was performed by sonography by one expert sonographer in phase 1 of the cohort in our research center. NAFLD was defined as hepatic steatosis in participants with no history of excess consumption of alcohol, drug-related steatosis or viral or hereditary steatogenic hepatitis. Anthropometric measures (height, weight) and blood pressure were measured by trained healthcare staff. A calibrated non-stretchable meter was utilized to measure the height of shoeless participants while standing with their heels and buttocks pressed against a wall. Subjects weight, after removal of excess clothing, was measured using a calibrated scale with a precision of 100 g. Blood pressure was measured with sphygmomanometer cuffs fitted to the arm circumference of subjects after resting, for at least 5 min in a sitting position in a quiet room. The systolic and diastolic blood pressures were determined based on the appearance and disappearance of Korotkoff sounds, respectively. The systolic blood pressure and diastolic blood pressure were calculated as the average of two measurements. Whole blood samples (10 mL) were taken from each participant using a serum separator tube, after 12 h fasting. The samples were exposed for 1 hour at room temperature to allow for clotting, then centrifuged rpm for 10 min and placed in 5000 cc straws with an aliquot using clean pipette technique. The vials of serum were immediately frozen at -80 °C in a freezer. To separate the plasma, the blood was collected in purple-topped EDTA tubes and centrifuged (2000 rpm) at 4 °C for 20 min. Subsequently, 1.0 mL of plasma was placed into 1.5 mL Eppendorf tubes using the clean pipette technique. Plasma samples for each subject was immediately frozen in a -80 °C freezer. Biochemical analysis was undergone by Auto-Analyzer BS200 (Mindray, Shenzhen, China). Pars Azmoon commercial diagnostic kits (Pars Azmoon Co., Tehran, Iran) included fasting blood sugar, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides were used.

Statistical analysis

Descriptive and analytical statistics were used to report the study findings. The incidence rate of fatal and non-fatal CVD events in participants with and without NAFLD was obtained and compared using the two-independent group proportion test. The simple and multiple Cox proportional models were conducted to determine the association between NAFLD in phase 1 of the cohort and the occurrence of fatal and non-fatal CVD events later during phases 1 and 2. The time of occurrence of fatal and non-fatal CVD events from 2009-2010 to 2016-2017 was considered to be the outcome for NAFLD as the predictor. The age at onset and history of DM in phase 1 of the cohort, as the main confounding variables, was used to explain the association

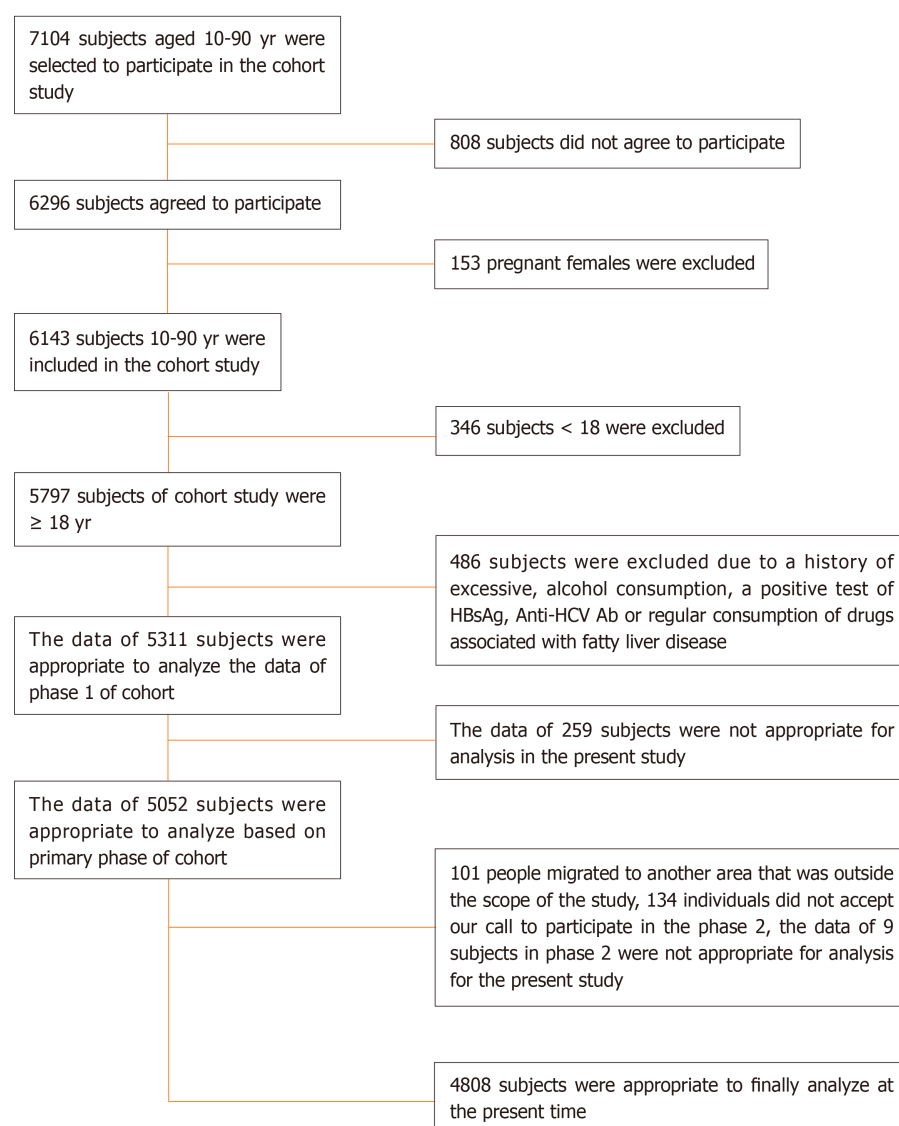


Figure 1 Flow diagram of phase I and phase II included and excluded participants. HCV: Hepatitis C virus; HBsAg: Hepatitis B surface antigen.

between NAFLD and CVD events. Hazard ratios and confidence intervals were reported at a significance level of 0.05 for all analyses. The software used for all statistical analyses was IBM SPSS version 21 (SPSS Inc., Chicago statistical software) and STATA version 12 (STATA Corp., TX, United States).

RESULTS

According to the sampling frame stratification used in this study, male study subjects numbered 2667 and female 2141. In these, NAFLD prevalence, as determined by sonographic data, was found to be 40.67% (95%CI: 38.89%-42.45%) and 43.58% (95%CI: 41.52%-45.65%) for males and females, respectively ($P = 0.0359$). The basic characteristics of the study population of males and females without and with NAFLD is shown in Table 1. The results indicate that all characteristics, except high-density lipoprotein cholesterol, were significantly higher in participants with NAFLD than those without NAFLD. The findings also showed that the prevalence of DM was 14.79% (95%CI: 12.77%-16.81%) within the males with NAFLD and 5.07% (95%CI: 4.04%-6.10%) in males without NAFLD ($P < 0.001$). On the other hand, the prevalence of DM in females with and without NAFLD was 27.27% (95%CI: 24.47%-30.08%) and 8.06% (95%CI: 6.55%-9.57%) respectively ($P < 0.001$).

Figure 2 shows the incidence (%) of fatal and non-fatal CVD events in individuals with and without NAFLD during the 7-year follow-up in males and females. In males,

Table 1 Basic characteristics of population study in people without and with non-alcoholic fatty liver disease

Characteristics	mean \pm SD		P value
	Without NAFLD	With NAFLD	
Men (<i>n</i> = 2667)	<i>n</i> = 1149	<i>n</i> = 1518	
Age (yr)	42.02 \pm 17.80	48.35 \pm 14.24	< 0.001
BMI (kg/m ²)	24.35 \pm 3.65	29.71 \pm 3.96	< 0.001
WC (cm)	84.82 \pm 10.39	99.57 \pm 9.65	< 0.001
DBP (mmHg)	73.76 \pm 12.18	80.72 \pm 12.07	< 0.001
SBP (mmHg)	114.13 \pm 14.65	121.81 \pm 15.71	< 0.001
FBS (mg/dL)	94.48 \pm 26.74	104.45 \pm 33.03	< 0.001
HOMA-IR	1.75 \pm 1.42	2.90 \pm 2.33	< 0.001
TG (mg/dL)	123.35 \pm 76.91	173.63 \pm 101.92	< 0.001
HDL (mg/dL)	45.65 \pm 11.51	40.31 \pm 10.94	< 0.001
LDL (mg/dL)	99.99 \pm 30.30	112.27 \pm 30.42	< 0.001
Women (<i>n</i> = 2141)	<i>n</i> = 934	<i>n</i> = 1207	
Age (yr)	37.88 \pm 15.09	50.20 \pm 12.37	< 0.001
BMI (kg/m ²)	27.01 \pm 4.83	33.11 \pm 4.76	< 0.001
WC (cm)	84.72 \pm 11.34	100.25 \pm 10.48	< 0.001
DBP (mmHg)	72.32 \pm 12.39	80.21 \pm 12.88	< 0.001
SBP (mmHg)	110.34 \pm 15.77	121.65 \pm 18.03	< 0.001
FBS (mg/dL)	94.97 \pm 30.36	115.22 \pm 49.96	< 0.001
HOMA-IR	2.20 \pm 1.62	3.43 \pm 3.13	< 0.001
TG (mg/dL)	115.25 \pm 67.10	173.92 \pm 118.78	< 0.001
HDL (mg/dL)	48.92 \pm 11.81	42.98 \pm 11.68	< 0.001
LDL (mg/dL)	104.15 \pm 30.62	116.51 \pm 31.06	< 0.001

NAFLD: Non-alcoholic fatty liver disease; BMI: Body mass index; WC: Waist circumference; DBP: Diastolic blood pressure; SBP: Systole blood pressure; FBS: Fasting blood glucose; HOMA-IR: Homeostatic model assessment for insulin resistance; TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein.

the incidence of non-fatal CVD events was significantly higher in individuals with NAFLD than those without NAFLD. Although the incidence of fatal CVD events was higher in individuals with NAFLD than those without NAFLD, it was not statistically significant. The incidence of fatal and non-fatal CVD events were higher in females with NAFLD than those without NAFLD; however, these differences were not statistically significant.

The Cox proportional models, used to determine the association between NAFLD and fatal or non-fatal CVD events as related outcomes yielded the following results (Table 2). While a positive simple association was detected between NAFLD and non-fatal CVD events in males (Hazard ratios = 1.606; 95%CI: 1.166-2.212; *P* = 0.004), no independent association was detected between them in the multiple Cox regression models. The results show no association between NAFLD and CVD events in females on the simple and multiple Cox proportional hazard models. Further results showed age and diabetes mellitus have an association with fatal and non-fatal CVD events. However, multiple cox model did not show any independent association between diabetes mellitus and fatal CVD events in women.

DISCUSSION

We evaluated the association between NAFLD and CVD events in a prospective study with a 7-year follow-up period. Fatal CVD events increased slightly in individuals (both males and females) with NAFLD compared to those without NAFLD, but this increase was not statistically significant. For non-fatal CVD events, although males with NAFLD developed a significant slightly higher number of CVD events in the 7-year follow-up compared to males without NAFLD, this was not significant in females. However, when we considered DM and age as potential mediators between

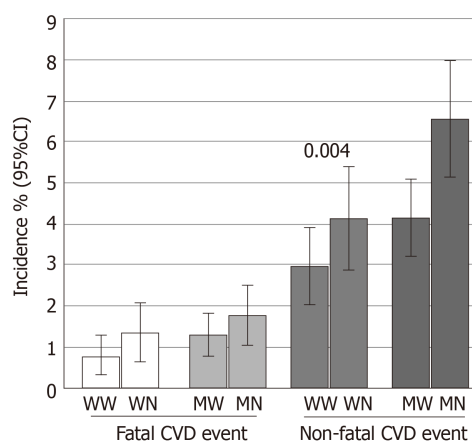


Figure 2 The incidence of fatal and non-fatal cardiovascular disease events in individuals with and without non-alcoholic fatty liver disease during a 7-yr follow up in men and women. CVD: Cardiovascular disease; WW: Women Without non-alcoholic fatty liver disease; MN: Men with non-alcoholic fatty liver disease; significant *P* value showed by score.

NAFLD and CVD events, no independent relationship was detected between NAFLD at the beginning of the study and fatal and non-fatal CVD events in the 7-year follow-up in either males or females. Stepanova *et al*^[17] reported an independent association between NAFLD and CVD events in the US population after a 14.3-year follow-up, although they found no association between CVD-related death and NAFLD, which is in line with the findings of the current study. Several other studies have investigated the association between NAFLD, with CVD events or outcomes. Chan *et al*^[18] found no association between NAFLD and prevalent Ischaemic Heart Disease (IHD) events among patients with DM. Hamaguchi *et al*^[19] suggested NAFLD as an independent predictor for CVD events. Zeb *et al*^[15] reported that NAFLD can be considered a risk factor for non-fatal cardiac heart disease independent of traditional cardiovascular risk factors. On the other hand, the study of Kim *et al*^[16] found no association between CVD death and NAFLD. Based on the evidence available to date, Targher *et al*^[20], suggested screening and surveillance strategies for cardiovascular diseases in patients with NAFLD, particularly those with steatosis. They went on so far as to claim that in many cases, people with non-alcoholic fatty liver will die of cardiovascular diseases before they die from an advanced liver disease^[20]. However, in a recent meta-analysis of matched cohort study of 18 million European adults, Alexander *et al*^[21] did not report any association between acute myocardial infarction or stroke and NAFLD, when the related analyses were adjusted based on the established cardiovascular risk factors. Although they emphasized on cardiovascular risk assessment in adults with NAFLD, unlike Targher *et al*^[20], they did not recommend strategies other than those suggested to the general population^[20,21]. Motamed *et al*^[13] found an association between NAFLD and 10-year CVD risk as estimated by both American College of Cardiology/American Heart Association and Framingham tools. In a more recent study, Han *et al*^[22], too, showed a significant association between NAFLD and CVD risk estimated *via* American College of Cardiology/American Heart Association tool. However, the risk assessment tools calculate the risks based on the data of established risk factors^[23,24]. Consequently, even assuming a very accurate prediction power for cardiovascular events, the risks calculated by these tools cannot show an independent relationship with such events independent of common cardiovascular risk factors.

Because there are common risk factors, such as obesity, diabetes mellitus, and insulin resistance, between nonalcoholic fatty liver and cardiovascular diseases, it is not easy to answer the question whether the association between nonalcoholic fatty liver and heart diseases is caused by these common risk factors or there is an independent relationship between them^[20]. However, as noted, our prospective study did not confirm this relationship.

Our results emphasize that age and DM can be considered major mediators in the development of non-fatal CVD events in males with NAFLD. In fact, a high prevalence of DM in individuals with NAFLD and a strong association between CVD and DM can increase the incidence of CVD events in patients with NAFLD. On the other hand, the association between age with both NAFLD and CVD events are another cause of the increased incidence of CVD events in patients with NAFLD.

Kunutsor *et al*^[25] showed that age has a critical role in the incidence of CVD events

Table 2 The results of Cox proportional hazard models on fatal and non- fatal cardiovascular events as outcome, and Non-alcoholic fatty liver disease, diabetes mellitus and age as potential predictors

Sex	Outcomes	Simple Cox proportional model			Multiple Cox proportional model		
		Wald	HR (95% CI)	P value	Wald	HR (95% CI)	P value
NAFLD							
In men	Fatal CVD events	0.963	1.345 (0.744-2.430)	0.326	0.104	0.903 (0.486-1.677)	0.747
	Non-fatal CVD events	8.400	1.606 (1.166-2.212)	0.004	3.723	1.384 (0.995-1.925)	0.054
In women	Fatal CVD events	1.570	1.694 (0.743-3.863)	0.210	0.002	1.178 (0.491-2.829)	0.714
	Non-fatal CVD events	2.327	1.416 (0.906-2.214)	0.127	0.063	0.941 (0.584-1.516)	0.802
Diabetes mellitus							
In men	Fatal CVD events	37.13	6.692 (3.631-12.334)	< 0.001	8.398	2.688 (1.377-5.247)	0.004
	Non-fatal CVD events	30.98	2.999 (2.037-4.415)	< 0.001	8.789	1.885 (1.240-2.867)	< 0.001
In women	Fatal CVD events	10.99	4.034 (1.769-9.201)	< 0.001	2.165	1.867 (0.813-4.290)	0.141
	Non-fatal CVD events	40.71	4.358 (2.773-6.850)	< 0.001	14.35	2.507 (1.558-4.032)	< 0.001
Age							
In men	Fatal CVD events	75.35	1.122 (1.094-1.152)	< 0.001	62.82	1.114 (1.085-1.144)	< 0.001
	Non-fatal CVD events	69.12	1.043 (1.033-1.054)	< 0.001	56.86	1.041 (1.030-1.052)	< 0.001
In women	Fatal CVD events	47.49	1.134 (1.094-1.176)	< 0.001	44.91	1.133 (1.093-1.176)	< 0.001
	Non-fatal CVD events	63.59	1.068 (1.051-1.085)	< 0.001	48.60	1.062 (1.044-1.081)	< 0.001

CI: Confidence interval; CVD: Cardiovascular disease; HR: Hazard ratio; NAFLD: Non-alcoholic fatty liver disease.

in patients with NAFLD in a 10.5-year follow-up. The simultaneous increase in the incidence of NAFLD and CVD events could be expected more often in older individuals than in younger individuals. The association between fatty liver and future CVD events was also attributed to dependence on insulin sensitivity by Pisto *et al*^[26]. The authors reported an independent relationship between fatty liver and CVD events, following adjusting for the confounding effects of consumption of alcohol, serum levels of low-density lipoprotein cholesterol, BMI and systolic pressure. However, when the confounding effects of the QUIKI index (as an index of insulin resistance) were removed, the statistically significant relationship between fatty liver and CVD events did not continue^[26,27].

Our study showed that there is no independent association between NAFLD and CVD events. The potential mediators of age and a history of DM were confounding variables for the association of NAFLD and the occurrence of new cases of CVD. This study had some limitations. One was the duration of the follow-up. The 84-month follow-up for participants without a history of a CVD event may not be adequate to monitor and establish full associations of CVD events between individuals with and without NAFLD. In addition, NAFLD in this study was evaluated using sonography rather than liver biopsy, which is regarded as the "golden standard". Although sonography is not optimal for the diagnosis of NAFLD, it is a non-invasive method that is ethically sensible for the diagnosis of NAFLD in research. Additionally, outcomes were based on objective and confirmed documentation by patients or close family. Hence, some patients with silent CVD events in this data collection strategy would not have been included in the study. However, we should consider that diabetes mellitus, as an important mediator between NAFLD and CVD events in our study, is a strong predictor for development of these silent events^[28,29].

It has been stated that age and DM are two strong potential mediators in the association between NAFLD and CVD events; thus, missed cases of CVD events in these two groups would attenuate the confounding effects of these mediators. This issue does not detract from the final conclusion that there was no independent association between NAFLD and CVD events in the 7-year follow-up of participants with no prior history of CVDs.

In conclusion, although we found a significant association between NAFLD and non-fatal CVD events in males, no independent association was detected between NAFLD and fatal and non-fatal CVD events in either males or females. Diabetes mellitus and age can be considered the principle mediators in this regard.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease worldwide. In addition to the relationship with liver related diseases and its complications, this condition may be associated with metabolic co-morbidities such as diabetes mellitus and dyslipidemia. Although previous studies have shown a positive association between cardiovascular disease (CVD) events and NAFLD, results showing an independent association between them are inconsistent.

Research motivation

The main topic in this study is to determine whether non-alcoholic fatty liver can lead to an increase in cardiovascular events independent of other potential risk factors. There is currently no consensus in this regard in the literature. Answering this question will help us determine if people with non-alcoholic fatty liver disease need more stringent cardiovascular interventions than the general population.

Research objectives

This study was conducted to determine if there is an independent association between NAFLD and CVD events.

Research methods

In this large prospective population based cohort study, valid outcome data of 4808 subjects were analyzed. These subjects had been followed up for seven years from phase 1, beginning in 2009-2010 to phase 2 during 2016-2017. The incidence of fatal and non-fatal CVD events were compared between people with and without NAFLD at the seven years follow up. Simple and multiple Cox proportional models were used to determine the association between NAFLD in the primary Phase of the cohort and subsequent fatal and non-fatal CVD events during follow-up.

Research results

The incidence of non-fatal CVD events in males with was significantly higher than in males without NAFLD. A positive association was demonstrated between NAFLD and non-fatal CVD events in males using the simple Cox proportional hazard model, but no independent association was detected between these in the multiple Cox models.

Research conclusions

Based on our results, Non-alcoholic fatty liver does not increase the risk of cardiovascular events independent of other risk factors. Diabetes and age may play a role as potential mediators. The presence of non-alcoholic fatty liver, apart from other cardiovascular risk factors, does not increase the need for stricter interventions to prevent cardiovascular disease than the general population.

Research perspectives

Further studies with a longer follow-up period may be needed in this area.

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