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Non-invasive assessment of liver fibrosis and portal hypertension in primary sclerosing cholangitis and primary biliary cholangitis: transient elastography, point share wave elastography and biomarkers.

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A zia Sina

Dolce sostegno, sempre e da sempre.

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1. Introduction

1.1 Primary cholestatic liver disease

Cholestasis is an impairment of bile formation and/or bile flow, which may clinically present with fatigue, pruritus and, in its most overt form, jaundice and signs of fat-soluble vitamin deficiencies. By convention, cholestasis is classified as intrahepatic or extrahepatic, and it is considered chronic if it lasts more than 6 months [1].

Differential diagnosis of cholestasis can be wide and sometimes challenging for the clinician. Careful anamnesis and physical examination, as well as a detailed pharmacological history are essential [2]. Imaging (ultrasound as first line, then Computed tomography and magnetic cholangiography) is required in order to exclude causes of mechanical obstruction of the bile ducts (i.e. gallstones, tumours, cysts, or strictures). Most chronic cholestatic diseases are purely intrahepatic and result from hepatocellular functional defects or from obstructive lesions of the intrahepatic biliary tract distal from bile canaliculi. A large number of patients are completely asymptomatic and identified by altered biochemistry on routine laboratory tests performed for other reasons. Serum alkaline phosphatase (ALP) and gamma-glutamyltranspeptidase (GGT) are early biochemical markers, followed, at more advanced stages, by elevated levels of conjugated bilirubin [1]. The currently known causes of intrahepatic cholestasis in adulthood are listed in **Table 1**.

Table 1. Causes of intrahepatic cholestasis in adulthood.

Hepatocellular cholestasis
Sepsis-, endotoxemia-induced cholestasis
Cholestatic variety of viral hepatitis
Alcoholic or non-alcoholic steatohepatitis
Drug- or parenteral nutrition-induced cholestasis
Genetic disorders: e.g., BRIC, PFIC, ABCB4 deficiency, intrahepatic cholestasis of pregnancy (ICP), erythropoietic protoporphyria
Malignant infiltrating disorders: e.g., hematologic diseases, metastatic cancer
Benign infiltrating disorders: e.g., amyloidosis, sarcoidosis hepatis and other granulomatoses, storage diseases
Paraneoplastic syndromes: e.g., Hodgkin disease, renal carcinoma
Ductal plate malformations: e.g., congenital hepatic fibrosis
Nodular regenerative hyperplasia
Vascular disorders: e.g., Budd–Chiari syndrome, veno-occlusive disease, congestive hepatopathy
Cirrhosis (any cause)
Cholangiocellular cholestasis
Primary biliary cirrhosis (AMA+/AMA−)
Primary sclerosing cholangitis
Overlap syndromes of PBC and PSC with AIH
IgG4-associated cholangitis
Idiopathic adulthood ductopenia
Ductal plate malformations: biliary hamartoma, Caroli syndrome
Cystic fibrosis
Drug-induced cholangiopathy
Graft vs. host disease
Secondary sclerosing cholangitis: e.g., due to various forms of cholangiolithiasis, ischemic choangiopathies (hereditary hemorragic telangiectasia, polyarteritis nodosa and other forms of vasculitis), infectious cholangitis related to AIDS and other forms of immunodepression, etc.

Adapted from EASL guidelines Management of cholestatic liver diseases 2009 [1].

Primary cholestatic autoimmune liver diseases are a rare aetiology of chronic liver disease that comprises primary sclerosing cholangitis (PSC) and primary biliary

cholangitis (PBC). These diseases are potentially progressive towards liver cirrhosis, portal hypertension and its complications, but nowadays lack of good prognostic stratifiers and have very limited therapeutical options [3].

The last decades have seen burgeoning clinical trial activity and, more recently, a growing interest in defining validated surrogates of clinical end-points both in PSC and PBC [4]. In particular, there is an increasing effort in developing new strategies and non-invasive tools for the diagnosis and the staging of cholestatic autoimmune liver diseases, such as novel serum markers (i.e. markers of extracellular matrix turnover) [5-10] and elastographic techniques [11-17]. The validation of point Share Wave Elastography (ElastPQ) for staging liver fibrosis in PSC and PBC and its utility in the detection of clinical significant portal hypertension has been the main subject of this research.

1.2 Primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) is a chronic, cholestatic liver disease characterized by an inflammatory and fibrotic process affecting both intra and extrahepatic bile ducts, which leads to irregular bile duct obliteration and formation of multifocal bile duct strictures (**Figure 1**) [18]. It is a progressive disorder that eventually develops into liver cirrhosis, portal hypertension and hepatic decompensation in the majority of patients [19, 20].

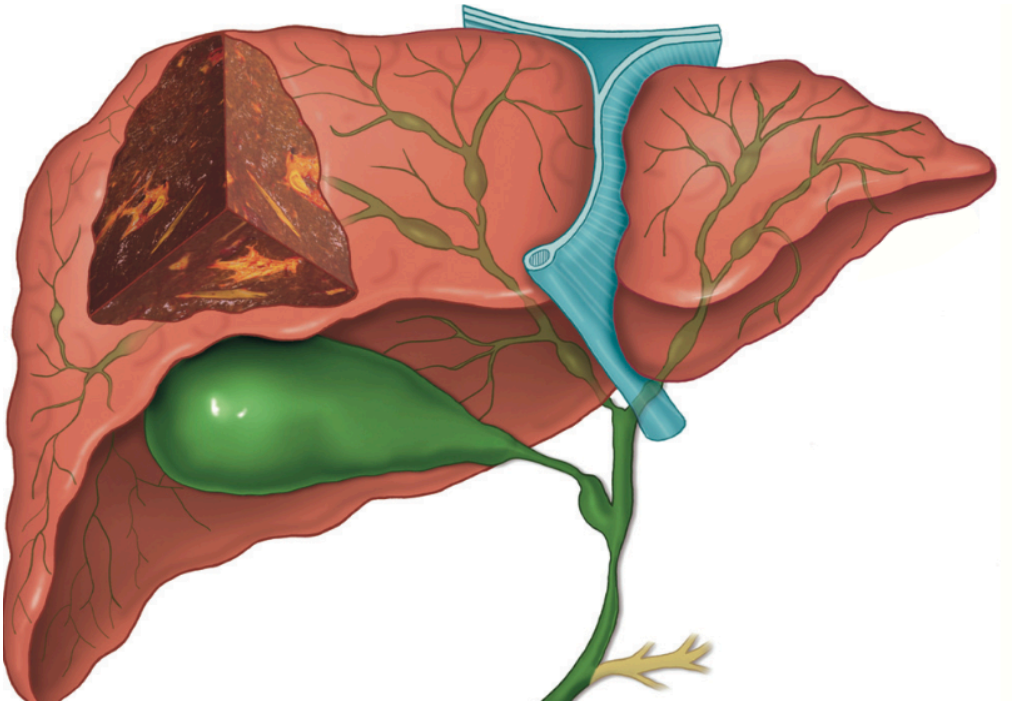


Figure 1. *Primary sclerosing cholangitis (adapted from Hirschfield et al., Lancet 2013)[18].*

1.2.1 Epidemiology

Affecting less than 200,000 individuals in the United States and less than 5 per 10,000 inhabitants in the European Union, PSC is considered a rare disease [21]. Prevalence rate is reported around 1 per 10,000 and an incidence in Northern Europe and the US ranges between 0.4 and 2.0 per 100,000 per year [22-24].

PSC prevalence seems to be higher in the Northern Europeans and Caucasians, with a geographical gradient increasing from South-East towards North-West and appears to be increasing over time [25-28].

The mean age at diagnosis is around 41 years and the disease is prevalent in male sex, with a male to female ratio of approximately 2:1 [20, 25, 27]. However, more recent data suggest that PSC may have a similar prevalence in both genders, but has significantly better outcomes in female patients (**Figure 2**) [29].

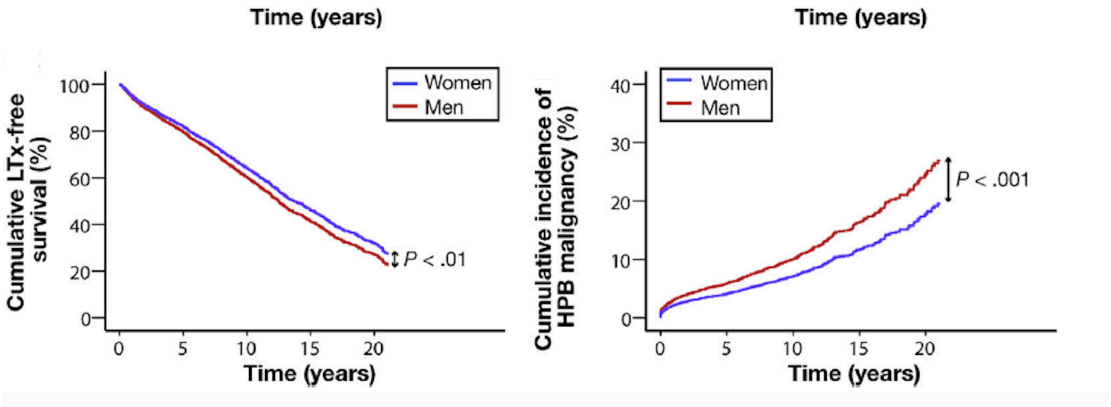


Figure 2. *Influence of gender on survival and prognosis in PSC (adapted from Weismuller, Trivedi et al., Gastroenterology 2017)[29].*

Up to 80% of PSC patients have concomitant inflammatory bowel disease (IBD), in the majority of cases ulcerative colitis (UC) [30] . On the other side, only a minority of patients with IBD is affected by concomitant PSC, which reaches a prevalence of 0.8 to 5.6% in UC and 0.4 to 6.4% in Crohn´s disease.

The diagnosis of PSC may precede or follow the one of IBD, which may even occur after liver transplantation for PSC, as PSC may present in an IBD patient even after colectomy [31-33]. Patients affected by Crohn's disease seem to have a less evolutive phenotype compared to those with UC or indeterminate colitis (**Figure 3**) [29, 34].

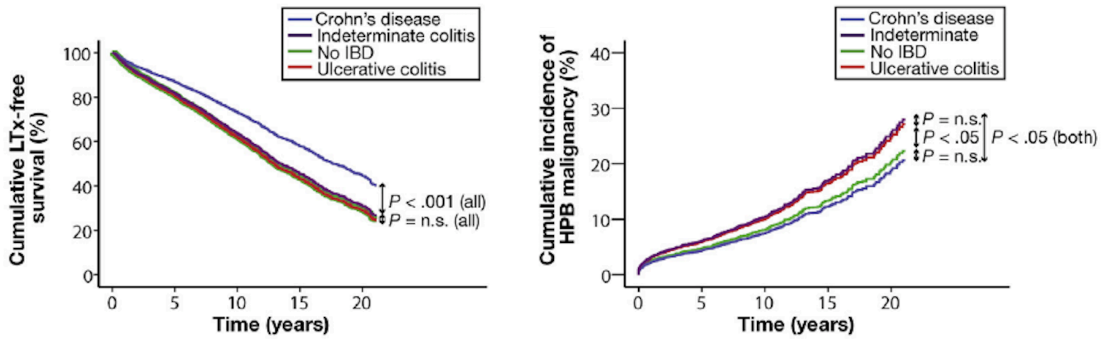


Figure 3. *Impact of inflammatory bowel disease (IBD) phenotypes on clinical outcome in PSC (adapted from Weismuller, Trivedi et al., Gastroenterology 2017) [29].*

Up to 25% of patients are affected by a concomitant autoimmune disease [35].

PSC patients are a heterogeneous population, affected by a wide spectrum of disease variants. In 6-16% of PSC patients the disease affects only the small intrahepatic bile ducts (small duct disease). This variant is characterised by a milder course of the disease, with fewer symptoms and a favourable prognosis compared to large-duct disease [36].

Other variant forms of primary sclerosing cholangitis include PSC with features of autoimmune hepatitis (AIH), once identified as "AIH overlap syndrome", which occurs in 7–14% of cases and is characterized by hepatocyte affection and biochemical and histological features of AIH [37, 38]. In this contest, immunoglobulin G4 (IgG4)-positive sclerosing cholangitis, characterized by increased serum levels of IgG4, massive infiltration of IgG4-positive plasma cells with storiform fibrosis and/or obliterative phlebitis in the thickened bile duct wall, which is frequently associated with autoimmune pancreatitis, might represent a separate entity [39, 40].

PSC is associated with an increased risk of biliary tract cancer, in particular cholangiocarcinoma (CCA), a malignant primary liver tumour originating from the bile ducts, with a generally poor prognosis and a high rate of mortality at 1 year [28, 41]. Generally rare, CCA is a major threat to PSC patients, with a reported 400-fold increased risk to develop CCA in PSC compared to the general population [42] and a lifetime incidence of up to 20% [43].

Hepatocellular cancer (HCC) seems to have a lower incidence than in other liver disease aetiologies [44, 45] and its risk mainly derives from the presence of established cirrhosis in these patients (*Saffioti et al., P15.07, EASL HCC Summit 2017*). Gallbladder stones and abnormalities are frequently observed in PSC. Up to 75% of the mass lesions or polyps observed are malignant, leading to reported gallbladder cancer

frequencies of 2.5% to 3.5% in the overall PSC population [46-50]. Pancreatic cancer may also occur in PSC, with a suggested increased incidence compared to the general population [51]. Furthermore, due to the high prevalence of IBD, PSC patients are also threaten by an increased risk colorectal cancer, which is five fold higher than in IBD without PSC and may occur at any time over the course of the disease [52].

1.2.2 Pathogenesis

Likely, PSC represents a "final common pathway" for multiple underlying mechanisms of bile duct injury, with still largely unknown aetiology and pathogenesis. Persistent portal inflammation, bile duct destruction, and periportal fibrosis lead to loss of bile ducts, disorganised ductular proliferation, and finally, cirrhosis [18]. There is no consensus yet whether the primary injury is caused by immune mediated mechanisms or biochemical aspects related to bile physiology/or alterations in cholangiocyte biology. There have been considerable advances in understanding the genetics of PSC, with the identification of more than 20 susceptibility genes and predominant HLA association [53-59]. However, environmental risk likely accounts for more than 50% [60]. Recently, there has been a renewed enthusiasm in the study of the linkage between the gut and the liver and the potential involvement of the gut microbiota in the pathogenesis in PSC [61]. Gut-derived antigens are potential triggers of adaptive immune responses, and several hypotheses linking inflammation in the gut and liver have been generated. The most acknowledged hypothesises an aberrant lymphocyte homing due to the abnormal expression of mucosal addressin cell adhesion molecule (MAdCAM)-1, normally restricted to the gut, on the hepatic endothelium. This leads to and ''enterohepatic circulation of lymphocytes'' and promotes the recruitment of

mucosal T cells to the liver via vascular adhesion protein (VAP)-1-dependent enzyme activity, contributing to portal inflammation in PSC [8, 62-64].

Several immune cells are found in the proximity of bile ducts of PSC, mostly T cells, macrophages and neutrophils, likely involved in the cross-talk with an activated cholangiocyte phenotype [65-67], and in the fibrogenetic process [68]. Anti-neutrophil cytoplasmic antibodies (ANCA) are frequently observed in PSC [69], and may reflect B cell responses to antigens of gut origin [70].

Cholangiocytes are protected against bile acid toxicity by several mechanisms, one of which is the presence of the so-called "bicarbonate umbrella", an $HCO3^-$ layer generated by an involvement of the Na^+-independent $Cl^-/HCO3^-$ anion exchanger and active Cl^- transporters [71, 72]. This mechanism appears to be deficient in PSC, and primary or secondary disturbances in bile homeostasis are hypothesised to contribute to the pathogenesis of the disease [60].

A schematic overview of the predominant processes involved in PSC pathogenesis is shown in **Figure 4.**

Importantly, the lack of understanding of PSC pathogenesis prevents the development of effective therapies.

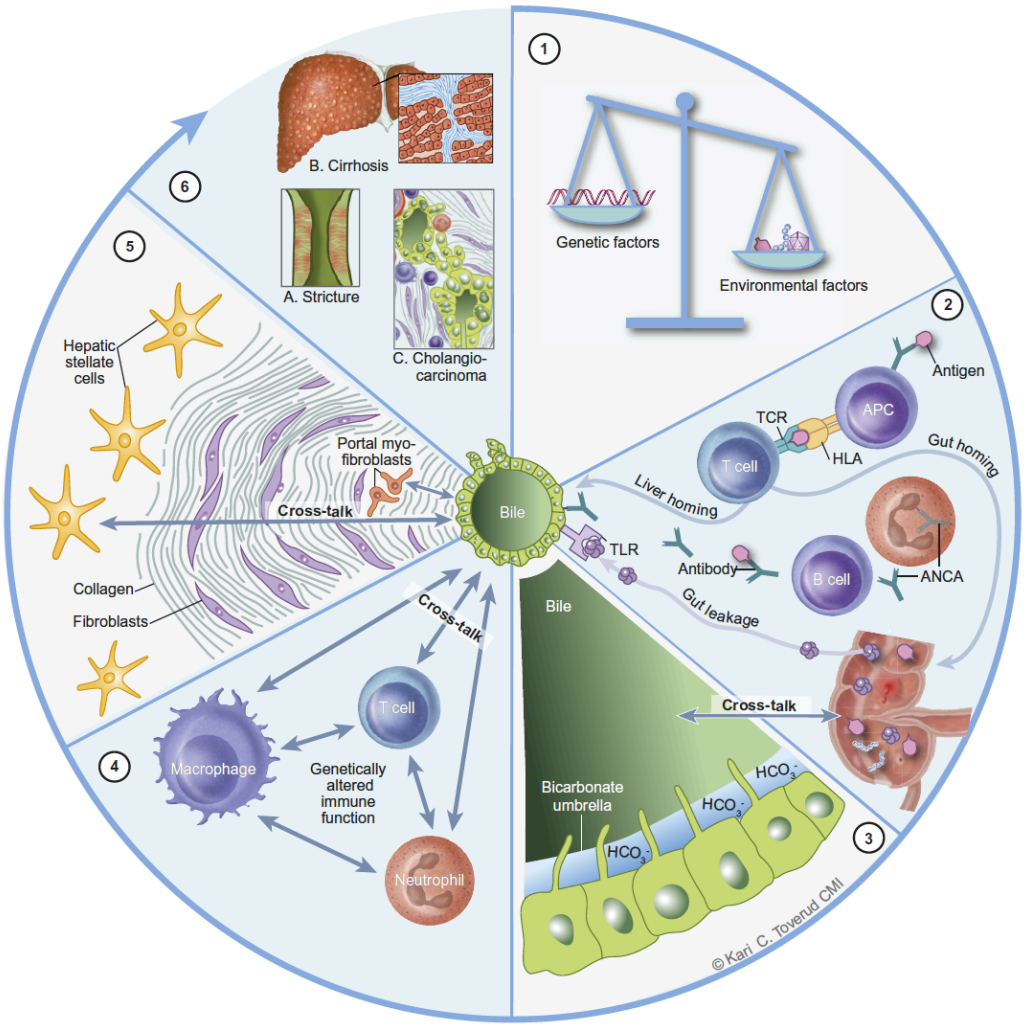


Figure 4. *Overview of the pathophysiopathology of primary sclerosing cholangitis (adapted from Karlsen et al. J Hepatol. 2017;67(6):1298-1323)[60].*

1.2.3 Natural History

The natural history of PSC is unpredictable and varies from longstanding indolent phenotypes to more aggressive and rapidly progressive ones (**Figure 5**).

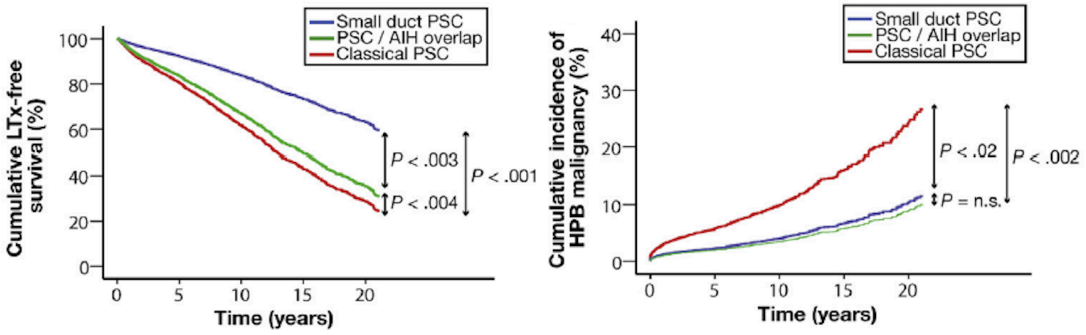


***Figure 5**. Impact of variant PSC sub-phenotypes on clinical outcome in PSC (adapted from Weismuller, Trivedi et al. Gastroenterology 2017) [29].*

Typical symptoms are pruritus, pain in the right upper abdominal quadrant, fatigue, weight loss, and episodes of fever and chills, even if about 50% of PSC patients are asymptomatic at the first presentation [73].

Disease course assessed by hepatic biochemistries and symptoms typically fluctuate. Similarly, IBD behaviour varies over time and does not correlate with the liver manifestations [60]. Liver disease progression is inevitable in most patients: the chronic biliary inflammation and the patchy peribiliary fibrosis lead to biliary duct strictures which cause regional cholestasis and, ultimately, cholestatic liver cirrhosis [18]. Despite the prolonged efforts, PSC lacks, at present, of an effective medical treatment [74], and liver transplantation remains the only curative therapeutic option for these patients [60].

The transplant-free survival time differs in population-based versus transplant centre cohorts, ranging from 13-21 years [28]. The risk of recurrence after liver transplantation in these patients reaches the 50% [75, 76].

1.2.4 Diagnosis

According to the current guidelines, the diagnosis of PSC is made in patients with elevated serum markers of cholestasis not otherwise explained, a cholangiography showing the characteristic bile duct changes with multifocal strictures and segmental dilatations, when secondary causes of sclerosing cholangitis have been excluded. [1, 77].

The pathognomonic histological lesion in PSC is a ‘‘onion skin” scar, referring to concentric layers of fibrosis circumferential to the cholangiocyte lining of the bile ducts (**Figure 6**) [18].

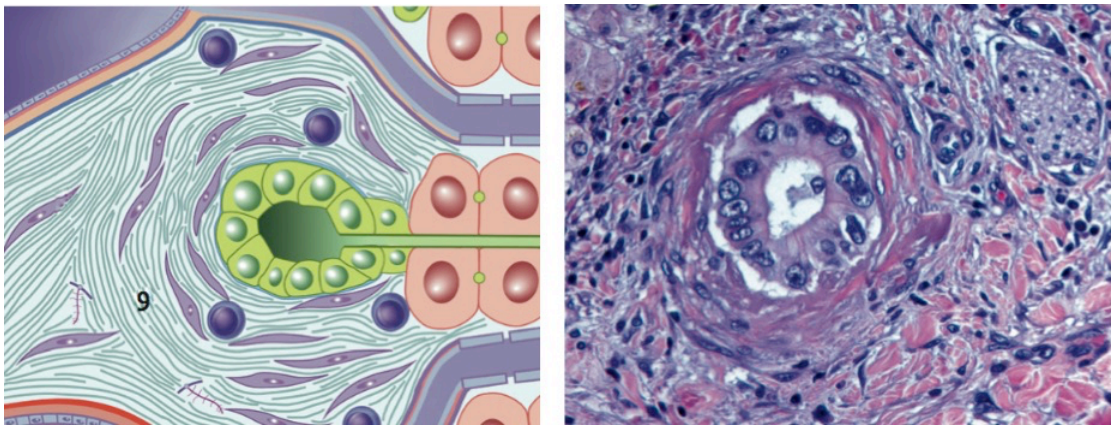


Figure 6. *Characteristic “onion skin” histological lesion in PSC (adapted from Hirschfield et al., Lancet 2013)[18].*

The distribution of the disease in the hepatic parenchyma is typically patchy, which may result in sampling variability on histological assessment of the liver [78]. Furthermore, the characteristic periductular lesions involve mainly medium-sized ducts and are frequently missed on liver sampling, thus affecting the diagnostic role of histology in PSC [79].

PSC causes fibrotic strictures and dilatations of the bile tree, which results in the

classical “beaded” appearance on cholangiogram [80]. Therefore, cholangiography by magnetic resonance imaging (MRCP) or endoscopic retrograde cholangiopancreatography (ERCP) are nowadays the gold standard for the diagnosis (**Figure 7**), while liver histology is no longer considered essential for establishing a diagnosis of PSC in patients with characteristic appearance on cholangiogram. However, a liver biopsy is recommended in the suspect of small duct PSC or alternative disease processes such as AIH [1, 77].

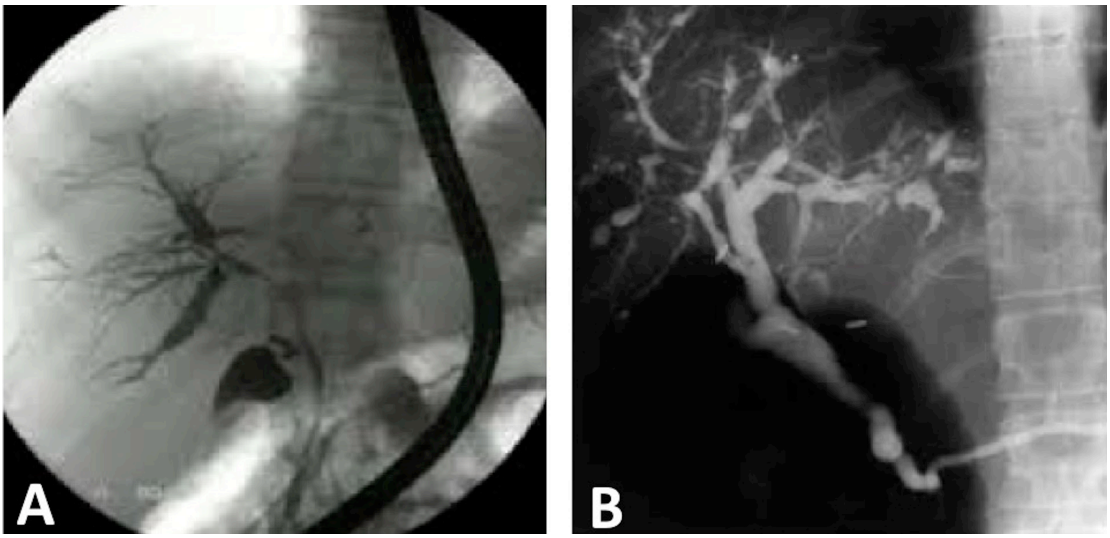


Figure 7. *“Beaded” bile ducts appearance in PSC on A) endoscopic retrograde cholangiopancreatography (ERCP) and B) magnetic resonance imaging (MRCP).*

1.2.5 Stratification and prognostication

Several prognostic models have been developed, over time, to assess and predict the natural history of patients with PSC [81-83]. These models have identified serum bilirubin, histological stage, age, haemoglobin, alkaline phosphatase, and the presence of hepatomegaly and splenomegaly as important prognostic indicators.

At present, the PSC revised Mayo risk score is the most widely used scoring system for stratifying patients with PSC and seems to provide valid survival information. The score incorporates serum bilirubin, albumin, AST, age and a previous episode of variceal bleeding and through a complex algorithm it estimates patient's survival at 1-4 years [84].

Other prognostic models for based on phenotypical and biochemical variables, namely the Amsterdam-Oxford score [85] and the UK-PSC score (*Good et al., Unpublished*) have been more recently conceived and validated to predict transplant-free survival.

A classification system based on intra- and extrahepatic ERCP findings in PSC conceived by the Amsterdam group has been proposed as a potential prognostic tool, but its applicability has been limited by its intrinsic invasiveness [86, 87].

As previously mentioned, liver biopsy is not routinely performed in PSC, however the degree of parenchymal damage and fibrosis seem to have an important prognostic value in PSC [88] and the results of various studies indicate that histology can adequately assess progression and regression of grade/stage of the liver disease [89-91]. Accordingly, histological changes have been included as an endpoint in several clinical trials in PSC [92]. Recent data demonstrated that histological scoring systems such as Ishak, Ludwig and Nakanuma are effective for measuring the degree of liver fibrosis in PSC, have a good reproducibility and can be used to predict clinical outcomes such as liver transplant-free survival and the development of cirrhosis-related events [92, 93].

Finally, quantitative liver fibrosis assessment by collagen proportionate area (CPA), a quantitative method to measure liver fibrosis using computer-assisted digital image analysis (DIA) of the proportion of collagen in liver tissue stained by the picro-Sirius red technique [94, 95], has been recently shown to correlate with the established

histological staging systems and to predict clinical events in PSC, assuming a potential role in the staging and the risk stratification in these patients (*Saffioti et al., Unpublished*).

In the last years, particular attention has been given to the development of non-invasive markers for stratification and prognostication of PSC [60].

A raised ALP, although non-specific, is the most characteristic biochemical finding of PSC. In the last decade, ALP has gained renewed attention with regards to its potential role as a prognostic marker. Alkaline phosphatase has in fact been used as an endpoint in every clinical trial on PSC with published data in the past 20 years [4].

According to the results of four retrospective cohort studies, including the two long-term post hoc analyses from the Scandinavian and the Mayo Clinic negative Ursodeoxicholic acid (UDCA) trials [96, 97], the normalization of ALP level or an improvement to <1.3-1.5 x ULN is associated with better clinical outcome and improved transplant- and CCA-free survival [98-101]. Thus, elevated levels of ALP seem to be a potentially good surrogate marker for disease progression and prognosis in PSC, even if further stratification, i.e. by stage of disease or biliary strictures distributions, may help to clarify prognosis in PSC [102]**.**

Recent findings in this context indicate a promising role of serological markers of fibrosis. Among these, the Enhanced Liver Fibrosis (ELF) score, a non-invasive marker of fibrosis based on three circulating markers of hepatic matrix metabolism (hyaluronic acid (HA), tissue inhibitor of metalloproteinases-1 (TIMP-1) and the pro-peptide of type III procollagen (PIIINP) [103]) has been recently demonstrated to be a potent prognostic marker in PSC for the prediction of transplant-free survival [5, 6]. More recent data have confirmed that extracellular matrix remodelling is elevated in PSC and, in particular, proved that markers of collagen formation such as PRO-C3 are

independently associated with clinical outcome [7].

Recent data highlighted the utility of non-invasive assessments of liver fibrosis by Vibration-Controlled Transient Elastography (VCTE) for staging and prognostication in PSC. Although the information is still limited in this disease, there is evidence that VCTE correlates well with histological fibrosis, being able to differentiate severe from non-severe fibrosis and cirrhosis, with high discriminative accuracy (AUROC 0.88) [14]. The diagnostic performance of VCTE was comparable to hyaluronic acid measurement, but superior to aspartate aminotransferase-to-platelet ratio index (APRI), Fibrosis-4 (FIB-4) and Mayo risk score in differentiating patients with significant or severe fibrosis from those without. VCTE has also been shown to have a prognostic value, with both baseline and delta elastography values correlating with clinical outcome. More precisely, patients with a liver stiffness higher than 11.2 kPa or a delta liver stiffness over 1 year higher than 1.5 kilopascal (kPa) are at high risk of hepatic complications in terms of death and liver transplantation [14, 15].

An alternative way of measuring liver stiffness is by magnetic resonance elastography (MRE). In the only published study in PSC, liver stiffness (LS) correlated with histological fibrosis stage in 20 patients and was predictive of hepatic decompensation in a series of 266 PSC patients [16]. The area under the curve (AUC) for detection of cirrhosis was 0.99, with a cut-off of 4.93 kPa. In addition, increased LS assessed by MRE was associated with the development of decompensated liver disease.

1.3 Primary biliary cholangitis

Primary biliary cholangitis (PBC), identified until a few years ago as "primary biliary cirrhosis" [104] is a chronic autoimmune cholestatic liver disease characterized by non-suppurative inflammation and destruction of the small to medium size intrahepatic bile ducts, classically associated with circulating anti-mitochondrial antibodies (AMA), which are disease-specific and target the E2-subunit of the pyruvate dehydrogenase complex (PDC-E2) located on the inner mitochondrial membrane (**Figure 8**) [77, 105-107].

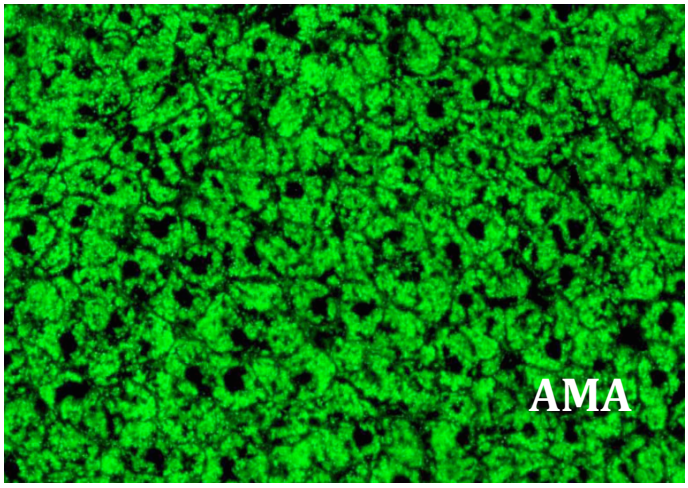


Figure 8. Anti-mitochondrial antibodies *(AMA) at immunofluorescence.*

The condition is generally slowly progressive but can lead to liver cirrhosis, portal hypertension and its complications and death [108]. PBC is often associated to symptoms such as fatigue, cholestatic pruritus, syndrome sicca and abdominal discomfort, which are often difficult to treat and can severely affect patients' quality of life [109, 110].

Although it is considered an autoimmune disorder, PBC does not respond to the conventional immunosuppressive therapies [3]. UDCA has been, for long time, the only drug approved for the treatment of the disease, with data showing biochemical and histological improvement [111-114]. However, about 30% of affected patients do not respond to the drug, and usually experiment a more severely progressive disease [115]. Furthermore, a recently published meta-analysis on clinical trials for PBC failed to demonstrated a clear beneficial effect on outcomes such as survival, liver transplant, development of cirrhosis or decompensation, of any of the pharmacological (including UDCA) treatments investigated until now [116]. Recently, obethicolic acid (OCA), a semi-synthetic hydrophobic bile acid analogue that is highly selective for FXR (a nuclear ligand-activated receptor abundantly expressed in tissues involved in the entero-hepatic circulation of bile acids), has been licensed for PBC patients with an inadequate response to UDCA, or for those intolerant to UDCA [108]. OCA has been shown to be effective in reducing ALP and bilirubin levels, but its use is complicated by frequent side effect (in particular exacerbation of pruritus and increase of serum cholesterol) [117, 118]. Furthermore the long-term effects of the drug are still unknown. Current available treatments, including off-label fibrates and budesonide [108] are of little efficacy in the more advanced stages of the disease and survival benefit has yet to be demonstrated.

1.3.1 Epidemiology

PBC affects mainly female sex (F:M=9:1) - although some recent data suggest an increasing male prevalence - with a mean age at diagnosis of 55 years [119, 120].

The clinical disease is rare and the estimate overall prevalence varies widely among epidemiological studies, ranging between 0.3–5.8 and 1.9–40.2 per 100,000 per year, respectively, with an increasing trend over time The estimated incidence in Europe is 1–2 per 100,000 persons per year [30, 121-124].

The diagnosis of PBC is established in the absence of symptoms in 20–60% of cases [125]. Thanks to the increased awareness of the disease and the improvement of the diagnostic tools, the number of asymptomatic patients diagnosed at an early stage is increasing over time [126]. Some patients may present AMA positivity without evidence of symptoms or liver tests abnormalities [127]. The overall prevalence of AMA antibodies is not known, but some studies reported estimates of 0.5–0.64% [124, 128]. About 16% of people with detectable AMA will develop overt disease at 5 years [129]. The prevalence of AMA positivity in first-degree relatives of PBC patients is increased compared to controls (13.1% and 1%, respectively), in particular regarding female sex [130]. In 5-10% of PBC patients, AMA is absent or found at a low titre (≤ 1:80) [131]. Antibodies against the major M2 components (PDC-E2) or antinuclear antibodies such as anti-glycoprotein 210 (anti-gp210) and/or anti-sp100 are detected in up to 30% of these patients and may correlate with prognosis [132, 133].

Compared to AMA-positive patients, AMA-negative PBC subjects present more severe histological damage [134] and concomitant non-hepatic autoimmune conditions [135, 136].

Other biochemical features typical of PBC are increased immunoglobulin concentrations, particularly IgM, which may be driven by epigenetic changes [137] and hyperlipidaemia, which can be detected in up to 80% of patients with PBC [138].

PBC patients present a higher prevalence of autoimmune diseases compared to the age and sex-matched population, and in particular Sjögren's/sicca syndrome; CREST

syndrome (Calcinosis, Raynaud's, Esophageal dysfunction, Sclerodactyly, and Telangectasias); scleroderma, Raynaud's disease and autoimmune thyroid disease [139].

Around 8–10% of patients exhibit biochemical ad/or histological features characteristic of AIH ("AIH-PBC overlap syndrome") and may benefit from immunosuppressive treatment [140].

Compared to healthy individuals matched for age and sex, patients with PBC have an accelerated bone loss due to reduced bone deposition, with osteopenia and osteoporosis diagnosed in about 30% and 10% of patients, respectively [126].

1.3.2 Pathogenesis

PBC is an idiopathic autoimmune cholestatic liver disease, in which genetic background and environmental triggers like infections, reproductive hormones, nutrition, vitamin D and chemical compounds play a combined role [107, 141-147].

The very high concordance rate for PBC (0.63) in monozygotic twins compared with dizygotic twins [141], the increased AMA rates in asymptomatic relatives, and disproportionate rates of disease in siblings of PBC patients, PBC family members and certain genetically-defined populations, as well as the more recent demonstration of the strong association of the disease with human leukocyte antigen (HLA) alleles produced from genome-wide association studies (GWAS) in large cohorts of patients support the hypothesis of a genetic cause of inherited risk [148-156]. Twenty-seven non-HLA loci associated with PBC susceptibility have also been identified [149] and epigenetic dysregulation events such as alteration in the DNA methylation and/or acethylation, as well as X chromosome monosomy and, more recently, alterations of cholangiocellular microRNAs have been widely described [137, 157-160].

The autoimmune mechanisms behind PBC seems to be related to a loss of immune tolerance to mitochondrial antigens (in particular the PDC-E2), possibly stimulated by infectious agents and/or chemical compounds through mechanisms of molecular mimicry and cross-reactivity, which lead to a pro-inflammatory burst against the cholangiocytes and a consequent progressive destruction of the bile ducts [161-163].

Defects in cholangiocyte bicarbonate secretion, and particularly an impaired activity of the anion exchanger 2 (AE2), have also been associated with the PBC phenotype. Physiologically, the intraluminal secretion of bicarbonate protects the biliary epithelium by the bile acids-related toxic apoptosis, while this protective alkaline "umbrella" is deficient in PBC [164, 165].

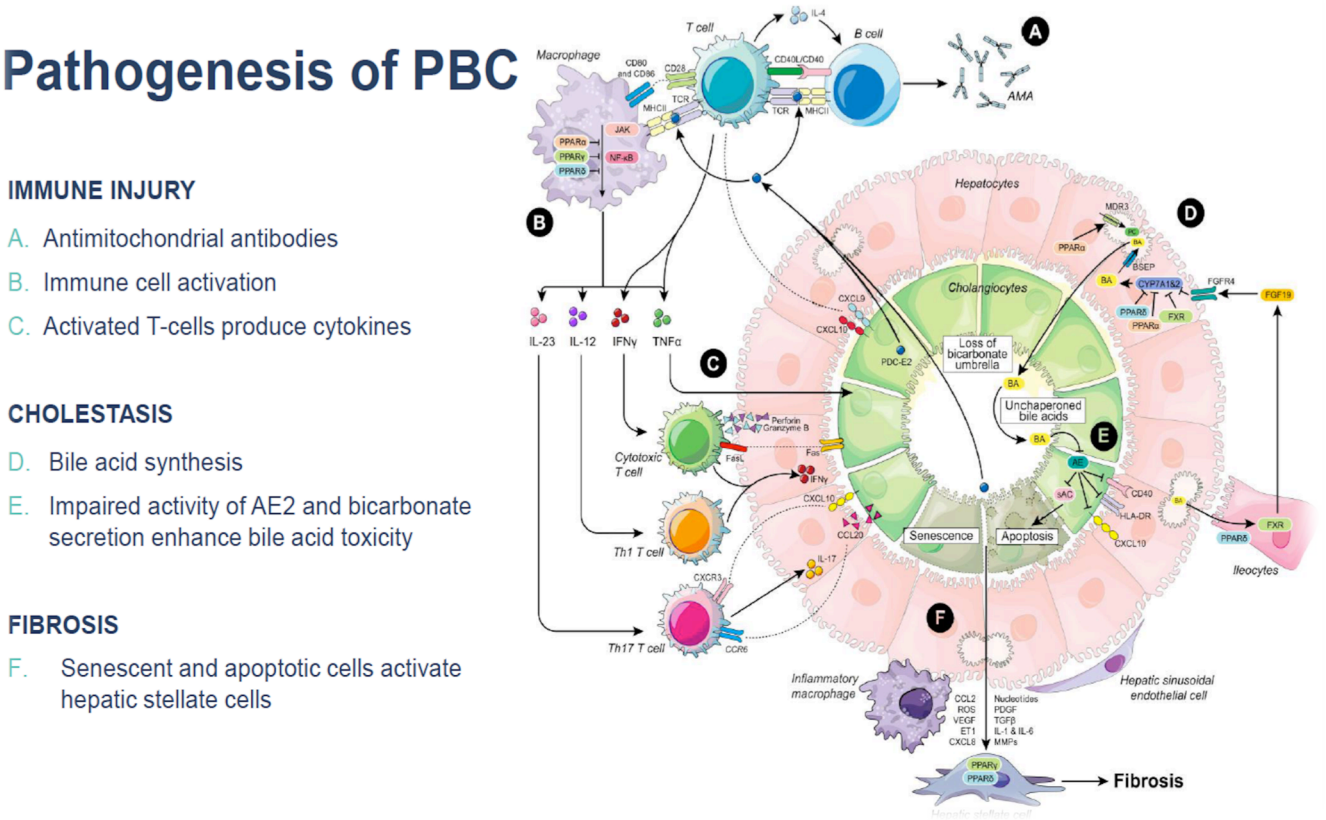


Figure 9. *Pathogenesis of Primary Biliary Cholangitis. Adapted from EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. J Hepatol 2017 [108].*

1.3.3 Diagnosis

Serological positivity for AMA is the hallmark for diagnosis of PBC, therefore a diagnosis of PBC can be made with confidence in adult patients with an otherwise unexplained cholestatic pattern of serum liver tests (elevation of ALP) for at least 6 months and the presence of AMA ≥ 1:40 and/or AMA type M2 in the serum [108], without the need of performing a liver biopsy. However, liver histology can provide some useful information on disease activity and stage [1]. Typical histologic findings in PBC include florid bile duct lesions with segmental degeneration of ducts and formation of poorly defined epithelioid granulomas (**Figure 10**) [1, 166, 167].

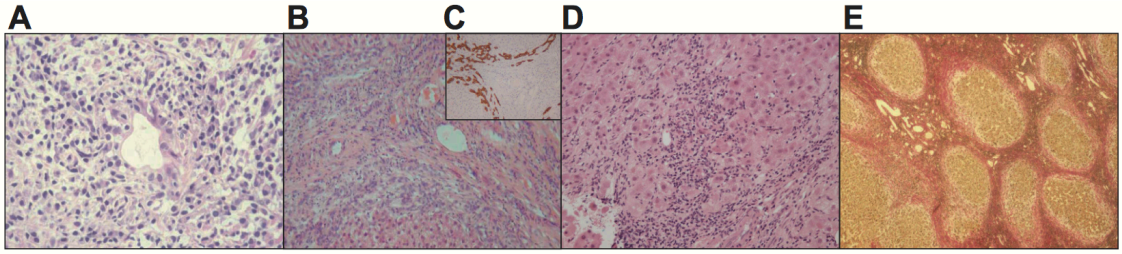


Figure 10. *Histopathologic features of primary biliary cholangitis. (A) Lymphocytic cholangitis; (B and C) Bile duct loss and ductular reaction; (D) Interface hepatitis; (E) Cirrhosis. (Adapted from EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis [108]).*

Immunofluorescence and/or specific anti-sp100/anti-gp210 testing by Western blotting or enzyme-linked immunosorbent assay (ELISA) are useful in the diagnosis of PBC in AMA negative patients [1, 129, 168-170].

Liver biopsy finds its indication for the diagnosis of PBC in patients in whom, in the absence of disease-specific antibodies, other causes of cholestasis have been excluded, when an additional or alternative processes such as AIH or non-alcoholic steatohepatitis (NASH) is suspected or in the presence of systemic/extrahepatic co-morbidities [1, 108].

1.3.4 Natural History

Despite the progression of the disease is generally slow and varies widely among patients, PBC is potentially evolutive towards liver cirrhosis and its complications.

PBC patients with cirrhosis are at risk of developing HCC. The incidence of HCC among patients with diagnosed PBC is estimated at 0.36 per 100 person years [171]. Results from a recent multicentre study confirmed that higher histological stage, male sex and inadequate response to UDCA are risk factors for HCC in PBC [172].

Male patients are usually diagnosed at a more advanced stage, have a poorer response to UDCA and generally present a worse prognosis, compared to female [120, 172, 173].

Untreated patients have a significantly shorter survival compared with a healthy population, with a median survival for symptomatic patients of 5-8 years from presentation, and a 25% chance of developing liver failure during this time [174-177].

Lower mortality rates have been reported in asymptomatic individuals, in which the median survival reached up to 16 years [174]. The 10-year survival for UDCA-treated patients, instead, is about 80% [108]. Furthermore, clinical trials have reported a positive effect of UDCA on reducing fibrosis progression and development of cirrhosis, in comparison to placebo or penicillamine [1, 108].

As in the other causes of cirrhosis, liver transplantation is the ultimate treatment for end-stage PBC. In PBC transplantation may also be considered in cases of intractable pruritus refractory to medical treatment [178, 179]. Post-transplant survival rates at 1 and 5-year is above 90% and up to 80–85%, respectively, and outcome is generally more favourable than for most other indications for liver transplantation, with need of re-transplantation in less than 10% of patients [180-182]. The rate of post-transplant recurrence has been traditionally stated to be around 20%. However recent data

obtained from a cohort in which liver biopsy was systematically performed at 1,5, 10 and 15 years showed an incidence of histological recurrence reaching the 60-70% at 15 years [183, 184]. Recurrent disease is generally benign and progression towards with graft failure is rare [185].

Thanks to his choleretic, cytoprotective, anti-inflammatory, and immunomodulatory properties, UDCA is nowadays the first-line therapy for PBC [108]. UDCA leads to biochemical improvement in the majority of treated patients and an improvement in transplant-free survival has also been reported [115, 186, 187] although not confirmed by some meta-analyses on the subject [116, 188]. The high rate of response to UDCA and the improvement of the diagnostic tools over time, which allow to recognise and therefore to treat the disease at earlier stages, have certainly had a positive impact on the natural history of PBC. As a result, a reduction of liver transplants for PBC has been registered over the last decades [180-182].

For a long time, UDCA was the only accepted pharmacological treatment in PBC, but the approval of OCA as second-line therapy and the recently published promising data on the positive effect of bezafibrate on biochemistry and itching [189] open new prospectives in the future management in PBC. Nevertheless, these combined treatment are mainly effective in patients with lower fibrosis and severity of cholestasis. Hence, the need to stratify patients and identify those at higher risk of progression and of developing complications, who may benefit from a tighter follow up and potentially need additional treatments.

1.3.5 Stratification and prognostication

Risk stratification is important to ensure that a personalised approach is provided to patients' care. The recently published European guidelines make a distinction between "dynamic" and "static" markers of risk stratification in PBC, according to whether response to treatment is considered [108]. In detail, demographics, symptoms, serological profiles, biochemistry, serum markers of fibrosis, liver stiffness measurement (LSM), histological features, and direct measurement of portal pressure are considered static markers that can be used at presentation or at any time during treatment.

A younger age at presentation (<45 years), male sex and the presence of symptoms are known to be associated with lower response to UDCA, a quicker progression and a poorer prognosis [172, 173, 190, 191].

PBC-specific ANA or anti-centromere antibodies seem to be associated with more severe phenotypes. However, given the paucity of longitudinal studies, the reliability of the serological pattern as a potential marker of prognosis needs further validation [113, 132, 192-196].

High bilirubin and low albumin are established predictors of poor outcome in PBC [106, 197, 198]. However, they become abnormal in the later stages of cirrhosis and hepatic dysfunction and therefore are not sensitive markers of progression earlier in the course of the disease. Similarly, the Mayo risk score (traditionally the most widely used prognostic system in PBC) [199], and the model for end-stage liver disease (MELD) score [200] are excellent predictors of death but, for the same reason, their utility in the early stages of the disease is questionable.

Histological staging of PBC (stages 1–4) has been proposed by Ludwig et al. [201] and Scheuer [202] according to the degree of bile duct damage, inflammation and fibrosis.

More recently, Nakanuma and colleagues have proposed a new grading and staging system for PBC, which takes into account particular features such as the presence of chronic cholangitis, hepatitis activity, and loss of bile ducts. A score of 0–3 is attributed to three histologic components: fibrosis, bile duct loss and deposition of orcein-positive granules and the total score identifies 4 different stages. The Nakanuma system seems to predict outcomes at 10 years more accurately that the traditional histology models [203].

As already discussed, despite some histological characteristics (such as the degree of lymphocytic interface hepatitis and the presence of ductopenia) have been shown to be independent predictors of histological stage progression, development of cirrhosis and/or major events in PBC [114], the use of liver biopsy for diagnostic purposes is no longer recommended. Furthermore, liver biopsy is subject to risk of complications and sampling error. PBC is, in fact, a "patchy" disease typically characterised by a not uniform distribution of fibrosis throughout the liver [204]. As a consequence, features of all four stages of PBC can co-exist simultaneously in a single bioptic specimen [1].

The direct measurement of the hepatic venous pressure gradient (HVPG) is highly correlated with the probability of death or liver transplant in PBC, and its reduction after 2 years of UDCA therapy may identify a subgroup of patients with good outcomes [205]. However, the emergence of non-invasive methods (i.e. elastography) for assessing portal hypertension indirectly, has drastically reduced the use of such an invasive procedure in routine practice.

LSM assessed by VCTE is one of the best surrogate markers for the detection of cirrhosis or severe fibrosis in patients with PBC [13, 206]. The best cut-offs validated against histology are 7.1, 8.8, 10.7 and 16.9 kPa for fibrosis stages ≥F1, ≥F2, ≥F3, and F=4, respectively. LSM has also shown to have an important value in predicting

outcomes in PBC. It has been reported that values ≥9.6 kPa are associated with a 5-fold increased risk of decompensation, liver transplantation or death. Variation of LSM from the baseline seems to be a stronger predictor of outcomes than LSM itself [13]. Furthermore, it has been recently demonstrated that patients with inadequate biochemical response to UDCA show a more progressive disease, which can be detected by worsening LSM (*Corpechot et al, Hepatology 2016:64:194A-195A*) and that LSM may provide added predictive value to newly introduced prognostic scores like the Globe score (*Corpechot et al, Hepatology 2016:64:S177-A178).*

It has been shown that ELF score can be used as a valuable tool to predict clinical outcomes in PBC, with a 3-fold increase in future complications for each one point increase [207]. In the only direct comparison between FibroTest (an algorithm of five fibrosis markers: alfa2-macroglobulin, apolipoprotein A1, haptoglobin, GGT, bilirubin), ELF and transient elastography, performed using histology as the reference method, it has been shown that both serum markers and VCTE performed with comparable diagnostic accuracy for the non-invasive staging of liver fibrosis in PBC [208]. More recently, APRI has been validated from different PBC cohorts as a predictor of adverse events, independently and additively of UDCA-response [209].

A number of definitions to define biochemical response to UDCA have been proposed, with Paris-I criteria showing the best performance in predicting outcomes (**Table 2**) [108]. These criteria are all based on the dynamic changes of standard serum liver tests under UDCA treatment (in most cases after 12 months from baseline), and established to predict histological stage progression or liver transplant-free survival. ALP and total bilirubin have been recently validated as robust pre-treatment predictors of UDCA response [210] and can be therefore used as surrogate markers of outcome for patients with PBC [108, 211].

In the attempt to improve the predictive value of the just-described prognostic tools based on dichotomous criteria, the Global PBC Study Group (http://www.globalpbc.com/globe) and the UK-PBC consortium (www.uk-pbc.com) have recently developed two scoring systems incorporating both measures of treatment response and parameters of disease severity [212, 213]. The GLOBE score and the UK-PBC score have shown comparable risk quantification and seem to be stronger predictors of death or liver transplantation compared to the Paris-I criteria [213].

Table 2. Criteria and scoring systems for assessing response to UDCA treatment.

Binary definitions	Time (months)	Treatment failure
Rochester	6	ALP ≥2 ULN or Mayo score ≥4.5
Barcelona	12	Decrease in ALP ≤40% and ALP ≥1 x ULN
Paris-I	12	ALP ≥3 x ULN or AST ≥2 x ULN or bilirubin >1 mg/dl
Rotterdam	12	Bilirubin ≥1 x ULN and/or albumin <1 x ULN
Toronto	24	ALP >1.67 x ULN
Paris-II	12	ALP ≥1.5 x ULN or AST ≥1.5 x ULN or bilirubin >1 mg/dl
Ehime	6	Decrease in GGT ≤70% and GGT ≥1 ULN
Continuous scoring	**Time (months)**	**Scoring parameters**
UK-PBC	12	**12 months:** bilirubin, ALP and AST (or ALT); **Baseline:** albumin and platelets
GLOBE	12	**12 months:** bilirubin, ALP, albumin, and platelet count; **Baseline:** age

Adapted from EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. J Hepatol 2017 [108].

Abbreviations: UDCA, ursodeoxycholic acid; ALP, alkaline phosphatase; ULN, upper limit of normal; GGT, gammaglutamyl-transpeptidase; AST, aspartate aminotransferase; ALT, alanine aminostransferase.

1.4 Staging liver disease

Since it has been shown that liver fibrosis is potentially reversible [214, 215], its early detection is fundamental in order to make therapeutic decisions, thus preventing, where possible, the progression towards more advanced stages of the disease [214, 215].

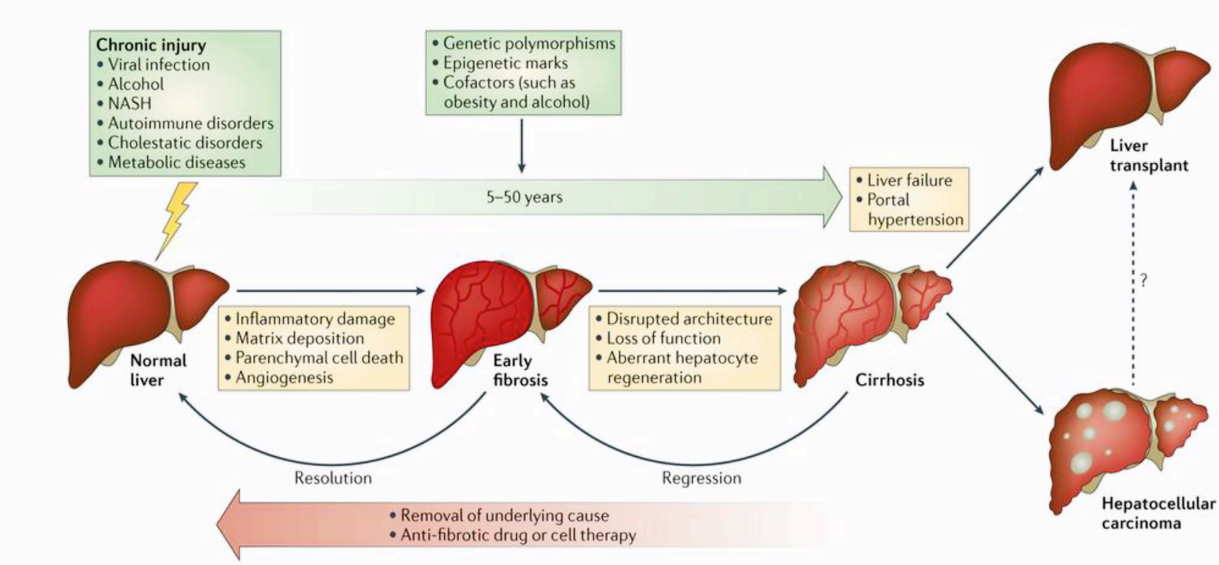


Figure 11. *Natural history of liver disease. (Pellicoro et al., Nat Rev Immunol 2014) [216].*

The Child-Pugh score and the Model for End-Stage Liver Disease (MELD) score are currently the most used to define prognosis by modelling hepatic dysfunction, but do not provide direct evidence of the stage or of the dynamic evolution of cirrhosis.

1.4.1 Portal hypertension and oesophageal varices

Portal hypertension (PH) is the most important consequence of cirrhosis and underlies its most severe clinical complications, including ascites, hepatic encephalopathy (HE) and bleeding from gastroesophageal varices [217].

Portal hypertension results from an increased intrahepatic resistance combined with increased portal (and hepatic arterial) blood flow. The increased intrahepatic resistance is the result of architectural distortion (fibrous tissue, regenerative nodules), endothelial dysfunction leading to intrahepatic vasoconstriction, and intrahepatic vascular shunting between afferent and efferent vessels of the liver [218, 219].

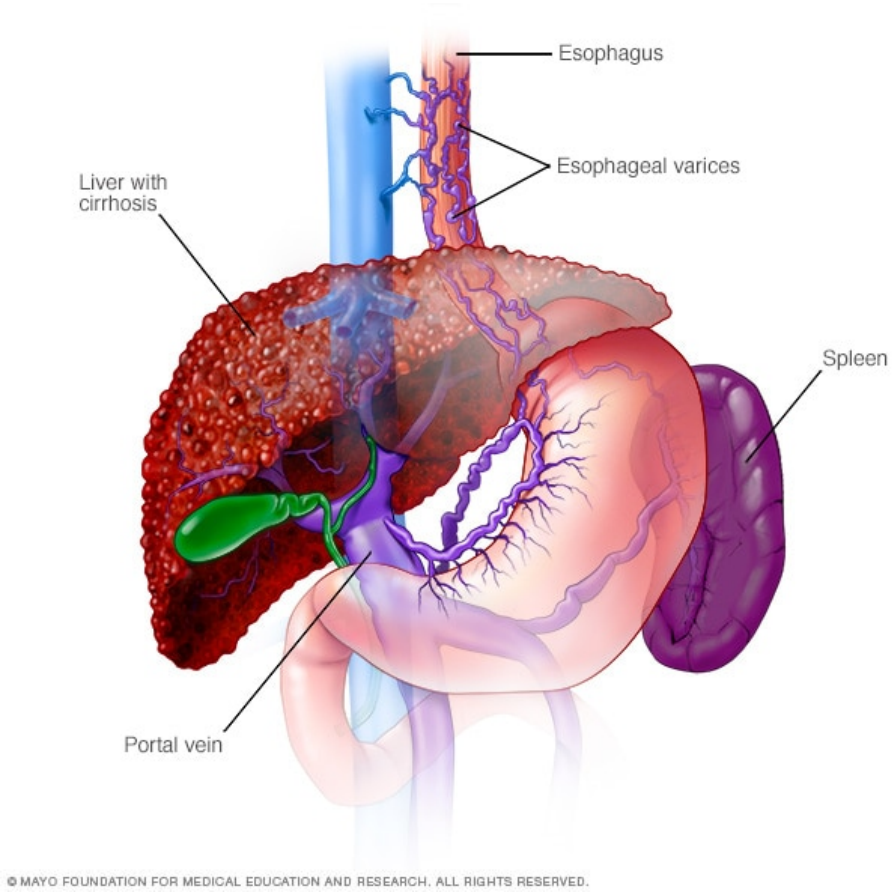


Figure 12. *Liver cirrhosis, portal hypertension and oesophageal varices.*

Hepatic-vein pressure gradient (HVPG) is a good surrogate marker of portal hypertension (normal values < 5 mmHg) and 10 mmHg is the identified threshold for the risk of developing varices and/or clinical decompensation ("clinically significant" portal hypertension) [81, 220-222].

The development of oesophageal varices, representing stage 2 of cirrhosis [218] (**Figure 13)**, is the result of the increased pressure in the portal system, the formation of portosystemic collateral circles (including haemorrhoids, recanalization of the umbilical vein and spleno-renal circles) and the angiogenesis stimulated by the VEGF and other angiogenic factors [81, 218, 223-226].

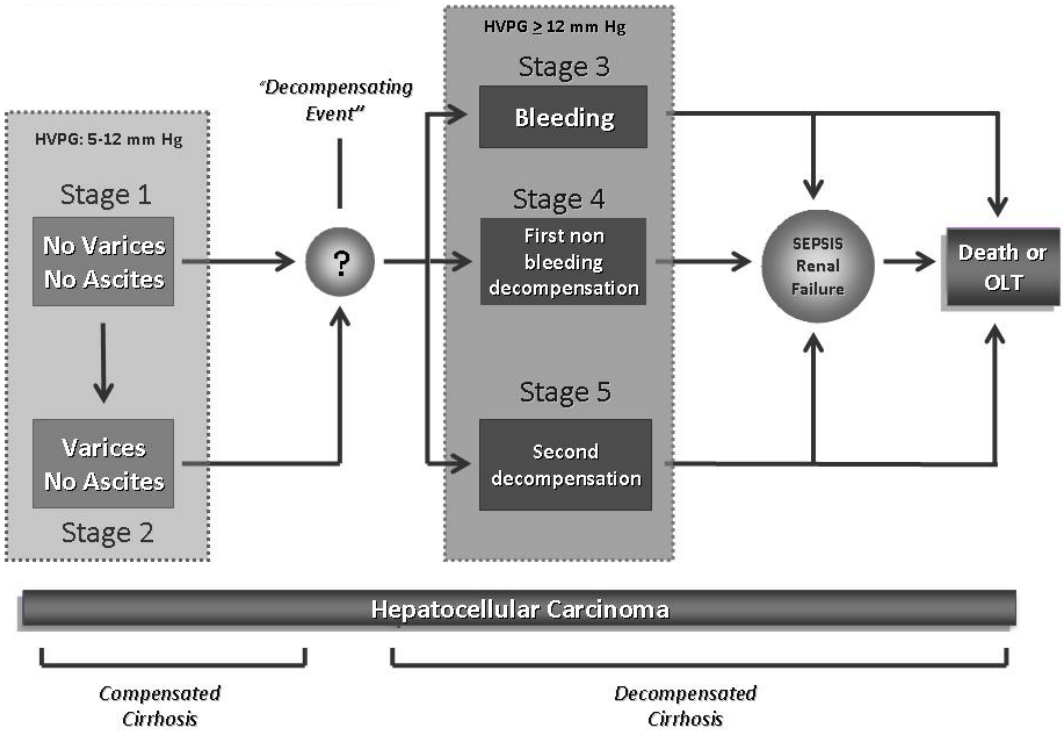


Figure 13. *Clinical Classification of Cirrhosis.*

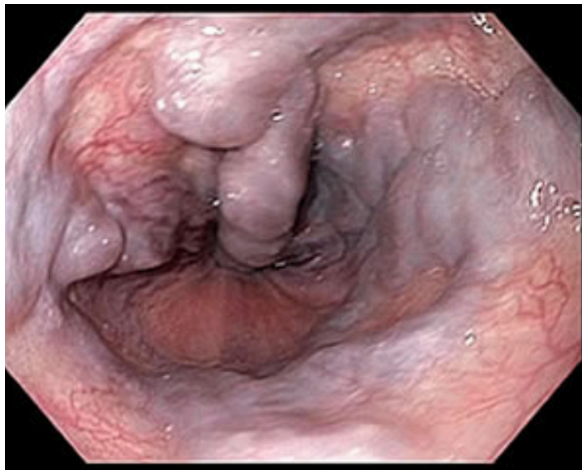


Figure 14. *Oesophageal varices.*

Treatment options for varices include non-selective beta-blockers (Propranolol or Carvedilol), irrespective of the size of the varices, or endoscopic band ligation, for medium or large varices.

Oesophageal varices bleeding is the most worrisome consequence of portal hypertension and represent a major threat to patients' life. Until a few years ago, the screening for oesophageal varices with oesophagogastroduodenoscopy (OGD) was recommended for all cirrhotic patients [227, 228].

Thanks to the progressive introduction of non-invasive methods for the diagnosis of cirrhosis, an increasing number of patients are diagnosed at an earlier stage of the disease, in which the prevalence of varices at risk of bleeding is relatively low (5-15%) [229, 230]. Screening these patients therefore results in a large number of unnecessary endoscopies. For this reason, guidelines for varices surveillance have been reviewed from a panel of experts at the Baveno VI Consensus workshop in 2015, which focused on "stratifying risk and individualizing care for portal hypertension''. The Baveno VI recommendations suggest that cirrhotic patients with a liver stiffness measurement (LSM) <20 kPa and a platelet count >150 × 10^9 cells/L can avoid screening endoscopy as their combination is highly specific for excluding clinically significant varices [228]. These criteria have been afterwards validated in large cohorts of patients with compensated advanced chronic liver disease [231, 232].

In order to improve their performance and further reducing the number of the saved endoscopies, the Baveno VI criteria have been expanded (platelet count >110 × 109 cells/L and LSM <25 kPa) and validated in a large multicenter retrospective study [233]. However, in most of the studies assessing Baveno VI criteria the number of patients with cholestatic liver disease was very limited [231, 233, 234], and their

performance (as well as the one of their expanded version) in PSC and PBC has only recently been validated in a multicentre study [235].

1.4.2 Elastographic techniques to measure liver stiffness and portal hypertension

Vibration-Controlled Transient Elastography (FibroScan®)

VCTE is performed by FibroScan® (F-TE), Echosens, Paris, France [236], a device consisting in a 5-MHz ultrasound transducer probe mounted on the axis of a vibrator which generates a completely painless vibration (with a frequency of 50 Hz and amplitude of 2 mm) leading to an elastic shear wave propagating through the skin and the subcutaneous tissue to the liver (**Figure 15**).

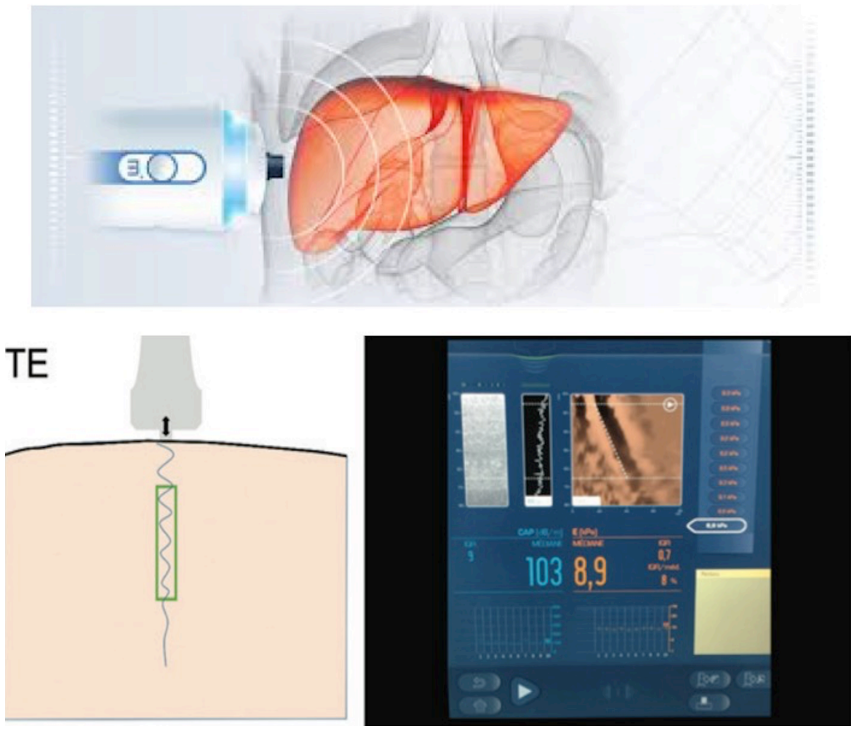


Figure 15. *Transient elastography performed Vibration-Controlled Transient Elastography (FibroScan®).*

The shear wave velocity, which is expressed in kilopascals (kPa), correlates with tissue stiffness: the stiffer the tissue, the faster the shear wave propagates [237].

F-TE has a well-established role in the non-invasive assessment of liver fibrosis from various aetiologies [238-244], including PBC [11, 13] and, more recently, PSC [14].

F-TE is a cheap and easy-to-learn technique, and thanks to its numerous advantages, including the wide availability and the possibility to perform the test at the bed-side, the short procedure time, and the possibility to get immediate results, it is widely used in clinical practice and it is considered the non-invasive standard for the measurement of LS [237]. F-TE has are well-validated quality criteria and is highly reproducible. However, the test is more accurate for the detection of cirrhosis than for the detection of significant fibrosis and performs better at ruling out than at ruling in cirrhosis [237].

The applicability of F-TE is limited in obese patients (problem partially resolved thanks to the introduction of the "XL" probe), but also in patients with narrow intercostal space, severe osteo-skeletal abnormalities (i.e. severe kyphosis), ascites. Furthermore, it is well known that cholestasis, inflammation, congestion (i.e. congestive heart failure), excessive alcohol intake, and recent food intake may influence FibroScan results, leading to an overestimation of liver stiffness values [237, 245]

Due to the lack of safety data in these groups of patients, the use of F-TE in pregnancy or in the presence of pacemaker is currently not recommended by the manufacturer.

Ultrasound-based elastography

From a physics point of view, elasticity (reciprocal of stiffness) is defined as the ability of a tissue of maintaining its shape after being challenged by a mechanical stress. This is an intrinsic characteristic of each tissue, and is expressed by the Young's elastic module. The main principle of quantitative ultrasound elastography is based on measuring the velocity of shear waves induced in a tissue by the tissue deformation (which is inversely related to the tissue stiffness) caused by the application of a mechanical stimulus such as vibration or an ultrasound impulse. The degree of the deformation is inversely related to the tissue stiffness, whereas the velocity of the shear waves is proportional [246, 247].

Over the last years, several ultrasound-based elastography techniques have been developed. Acoustic radiation force impulse imaging (ARFI) is the physical principle standing behind all the ultrasound-based elastography methods. The enforced acoustic impulse causes displacement of the liver tissue and induces shear waves that travel perpendicularly to the direction of the original acoustic impulse. In 2D-shear wave elastography (2D-SWE), the velocity of the multiple shear waves is tracked by the ultrafast ultrasound transducer (receiver) and this information is used to construct the 2D image of the examined region of interest, in which each pixel of the image represent the absolute value of the shear wave velocity at that specific point in real time. This allows the operator to visually control the quality and reliability of the elasticity maps [237]. Because in 2DSWE the elastogram is displayed in real time, one single stiffness measurement might suffice, in theory. However, even with this method experts and international guidelines recommend to perform 3-5 acquisitions and calculate median value and IQR [248].

In point shear wave elastography (pSWE) (**Figure 16**) the basic principle is the same, but 2D image is no not constructed. Instead, the velocity of the generated shear waves is tracked in a small box (region of interest), without making the elastogram visible [237]. In pSWE, the operator does not have possibility to control the quality of the elasticity maps and the resultant stiffness measurements, therefore quality criteria rely exclusively on the statistical calculation of the median value of the stiffness measurement, which should have low dispersion around the median value as expressed by IQR<30% [248].

Among pSWE methods, there are now several commercially available solutions such as Virtual Touch Tissue Quantification (VTQ®), by Siemens, which is most usual and wrongly called ARFI (again: ARFI is the physical principle behind US elastography methods), ElastPQ® by Philips, and some others. Measurements are reported either as metres/second or as kPa, according to the settings.

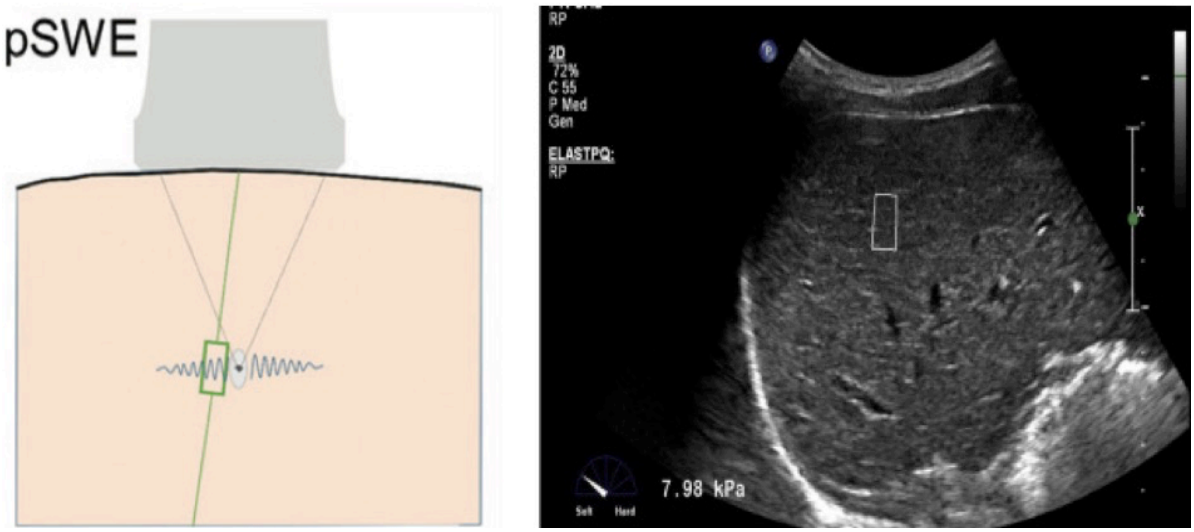


Figure 16. *Transient elastography performed with point shear wave elastography (pSWE).*

Similar to F-TE, pSWE is more accurate for detecting cirrhosis than significant fibrosis [249], but it as a wider applicability, allowing to measure stiffness also in patients with ascites or obese. As for F-TE, results may be influenced by food intake and inflammation, which lead to an overestimation of liver fibrosis [237].

Point Share Wave Elastography has been successfully applied in patients with viral hepatitis and non-alcoholic fatty liver disease [250-252], but data about its performance in rarer aetiologies such as PSC, PBC and AIH are limited [253].

Magnetic resonance elastography

Magnetic resonance elastography (MRE) uses a modified phase-contrast method incorporating motion-encoding gradients to image the propagation characteristics of the shear wave in the liver induced by a vibrating compression device [254].

Advantages of MRE include its ability to analyse almost the entire liver and its good applicability in patients with obesity or ascites. However, it cannot be used in patients with iron overload (due to technical issues), and the high costs and the logistic needs (including long times requirement) currently limit its employment in routine clinical practice [237].

Spleen stiffness

Over the last years, the spleen has gained a renewed attention as a potential surrogate marker of portal hypertension. It is well known, in fact, that in patients with cirrhosis the spleen undergoes enlargement and changes mostly related to portal hypertension

[245, 255].

Spleen diameter, in combination with platelet count (platelet count/spleen diameter ratio) has been validated in multiple studies and found to be the most accurate non-invasive test for the prediction of the presence of oesophageal varices in a recently published meta-analysis on the argument [256].

Spleen-stiffness measurement (SSM) has been recently proposed as a novel parameter to better mirror portal hypertension as compared to LSM [257]. It has been shown, in fact, that SSM performs better than LSM in predicting the presence of oesophageal varices and improves the performance of the Baveno VI criteria alone, for the detection of high-risk oesophageal varices [258, 259].

SSM has low applicability in normal-sized spleens (splenic parenchymal thickness $<$ 4 cm). Another limitation, when the measurements are obtained with TE, is the upper limit value of 75 kPa, which limits risk stratification above this threshold [255].

2. Aims and research plan

A cross sectional study was carried out with the following aims:

- To validate pSWE as represented by ElastPQ as a non-invasive method for the assessment of liver fibrosis and staging in patients with PSC and PBC.
- To evaluate the correlations of LSM by ElastPQ with the currently validated scores and non-invasive markers of liver fibrosis and with F-TE in a cohort of patients affected by PSC and PBC.
- To assess the performance of LSM and SSM as performed by ElastPQ in detecting the presence of clinically significant portal hypertension in PSC and PBC.

3. Materials and methods

3.1 Participants Identification and Recruitment

This research was conducted at a Hepatology tertiary centre, the Royal Free Hospital London, between November 2015 and March 2018.

Inclusion Criteria

1. Confirmed diagnosis of PSC, PBC, according to the validated criteria [1, 77, 108].
2. Age > 18 years.
3. Signed informed consent.
4. Optimal period of fasting of at least 3 hours.

Exclusion criteria

1. All cases of secondary sclerosing cholangitis, which include: congenital biliary tree abnormalities, previous biliary surgery, bile duct carcinoma, HIV cholangiopathy, sarcoidosis, graft-versus-host disease and drug reactions, IgG4 cholangiopathy.
2. Other concomitant liver diseases, except for AIH overlap syndrome (quiescent) and fatty liver (not-NASH).
3. Autoimmune overlap syndrome flare.
4. Aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) values higher than 5 times the upper limit of normal (ULN) [237, 248].
5. Patients undergone liver transplantation.
6. Patients with current dominant stricture (cholangiographically defined as a stricture < 1.5-mm diameter in the common bile duct, or < 1 mm in the left or right main hepatic ducts [1]).
7. Decompensated cirrhosis.
8. Moderate to severe ascites.
9. Patients with current bacterial cholangitis.
10. Severe obesity (body mass index ≥ 40 kg/m^2).
11. Medical history of cardiac disease and pulmonary hypertension.
12. Patients with congestive liver disease [237, 248].
13. Current pregnancy, in female patients.
14. Patients with cardiac pacemaker or implantable cardioverter defibrillator (ICD).

Patients meeting the above criteria were invited to participate to the study, and were enrolled after having read the information sheet and signed the consent form.

On the enrolment date, patients underwent:

- Baseline clinical assessment,
- Routine bloods collection (full blood count, biochemistry, clotting),
- Liver stiffness measurement with F-TE and pSWE,
- Spleen size measurement with ultrasound,
- When possible, spleen stiffness measurement with pSWE.

F-TE and pSWE were consecutively performed by expert operators in liver elastography. pSWE was performed first, followed by F-TE as the reference method.

Clinical scoring systems and non-invasive scores of severity of liver disease were calculated on the basis of baseline data (described below).

Demographic data were acquired and clinical information was retrospectively obtained from patient's clinical records.

Upper gastro-intestinal (GI) endoscopy for varices screening was performed in patients with established liver cirrhosis, as part of the routine clinical practice. High-risk varices were defined as grade ≥2 oesophageal varices, or any varix with a red colour sign. Only endoscopies performed within 12 months from the liver stiffness measurements were considered in the analysis.

When available, liver histology (METAVIR score) performed within 12 months from the visit date was considered for fibrosis staging.

3.2 Methodology of non-invasive assessment of disease severity.

3.2.1 Ultrasonography and Elastography

Abdominal Ultrasonography – Prior to every elastographic measurement an upper abdominal ultrasound assessment was carried out to evaluate the presence of biliary dilatation, gallstones or polyps, the patency of the portal vein and hepatic veins as well as the presence of ascites and spleen size in terms of length and area.

Each patient was placed in the supine position and a conventional 3.5 MHz probe was used. Spleen longitudinal cross-sectional area and bipolar diameter (crossing the spleen hilum) were measured. Splenomegaly was defined as a spleen length greater than 120 mm and spleen area greater than 45 cm^2 [260].

Liver transient elastography was performed using the FibroScan® (Echosense, Paris), placing the transducer perpendicularly to the skin in the right intercostal spaces, with the patient lying in dorsal decubitus with the right arm in maximal abduction. The test was considered valid when at least 10 successful acquisitions with a success rate of at least 60% and an IQR (range of interquartile) ≤ 30% were obtained.

Liver point shear wave elastography was performed using the Affiniti 70 G ultrasound system (Philips Healthcare, Bothell, WA, USA) with a convex C5-1 broadband probe and the ElastPQ® software. Patients were placed in supine position, with the right arm in maximal abduction to widen the intercostal spaces and optimize images acquisition. Measurements were acquired while patients were holding their breath for a few seconds after expiration, by placing the transducer between one of the intercostal spaces to secure an appropriate acoustic window. The preferred target area

of liver parenchyma was in the upper right lobe, usually segment V [261]. After obtaining an adequate B-mode liver image, the region of interest (ROI) was targeted by placing the 0.5 x 1 cm box-cursor on the liver parenchyma avoiding large vessels and bile ducts, with the upper edge of the box placed 1.5–2.0 cm away from the Glisson capsule [262]. Ten consecutive measurements were acquired. A reliable result was defined as an examination in which the success rate was higher than 60%. The final data were displayed in kPa [263].

Spleen point shear wave elastography was performed using the same technique, after obtaining a good visualization of the organ, placing the transducer perpendicularly to the skin in the left intercostal spaces, with the patient lying in dorsal decubitus with the left arm in maximal abduction. ROI was positioned 1-2 cm below the splenic capsule in correspondence of the middle lobe and 10 consecutive measurements were acquired.

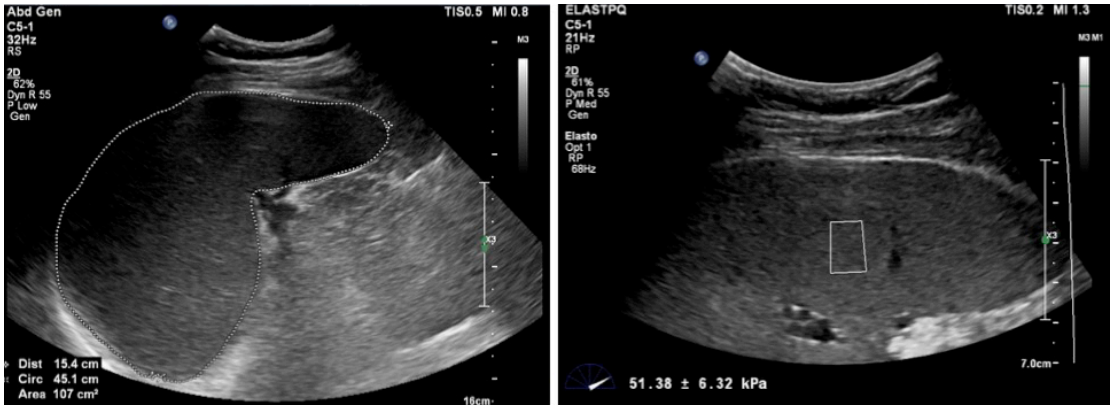


Figure 17. *Spleen size measurement and transient elastography performed by ElastPQ.*

3.2.2 ELF Testing

When an ELF tests was available (performed from serum sample obtained on the date of the elastographic assessment, in the context of other clinical studies, using ADVIA Centaur® XP system – Siemens Healthcare Diagnostics Inc. – Tarrytown, NY, USA), the ELF score was calculated with the published algorithm, combining TIMP-1, HA, and PIIINP values: ELF score = 2.278 + 0.851 ln(CHA) + 0.751 ln(CP3NP) + 0.394 ln(CTIMP1) [264].

3.2.3 Prognostic scores

The ***PBC Mayo risk score*** and the ***Revised PSC Mayo risk score*** were calculated for all PBC and PSC patients, respectively using the specific algorithms [84, 199], considering blood tests performed on the day of the elastographic measurement.

Child-Pugh score and ***MELD score*** [200] were calculated for all the patients, and the recent Oxford-Amsterdam score [85] was obtained for PSC patients.

3.2.4 Scores of fibrosis

The following scores, ratios and indices were calculated for all patients, based on the serum tests results on the day of elastography:

- **AST/platelet ratio index (APRI) =** *(AST/TopNormAST)*(100/Platelets) [265]*
- **Aspartate aminotransferase-to-alanine aminotransferase ratio** = *AST/ALT [266]*
- **FIB-4** = *(age [yr] * AST) / ((Platelets [x10^9/L]) * (ALT)(1/2)) [267]*

- **King's score** = *Age (years) * AST (IU/L) * INR / Platelets (10^9/L) [268]*
- **Fibrosis index** = *8.0-0.01 * Platelets (10(3)/microliter) - Alb (g/dl) [269]*
- **Lok score** = $e^{(LogOddsLok)} / (1 + e^{(LogOddsLok)})$, *where LogOddsLok = (1.26 * AST/ALT) + (5.27 * INR) - (0.0089 * Platelets) - 5.56 [270]*
- **Fibro Q** = *[(10 * age (years) * AST * PT INR)/ (Platelets * ALT)] [271]*
- **Göteborg University Cirrhosis Index (GUCI)** = *(AST/TopNormalAST) * INR * 100/Platelets*
- **Bonacini score** = *Platelets ($x10^9$/L)+ALT/AST (IU/L)+INR* [272]
- **LSPS***: liver stiffness measurement x spleen diameter/platelet count [273]*
- ***SSPSA:*** *LS * spleen area/Platelets (Rosselli & Roccarina, Unpublished)*

3.3 Statistics and data analysis

Continuous variables were expressed as mean ± standard deviation or median and interquartile range (IQR), as appropriate according to distribution (Kolmogorov-Smirnov test). Dichotomous variables were expressed as frequencies and percentage. Chi-squared test was used for percentages comparison. T-Student and Mann-Whitney tests were used to compare continuous variables according to their normal or not normal distribution, respectively.

The correlation between quantitative variables was assessed by Spearman's ranking test. Lin's concordance correlation coefficient (CCC), which can been expressed as the product of Pearson's r (the measure of precision) and the bias-correction factor (Cb,

the measure of accuracy), was used as a measure of the concordance between F-TE and ElastPQ liver elastography. CCC ranges in values from 0 to +1. Agreement was classified as poor (0.00–0.20), fair (0.21–0.40), moderate (0.41–0.60), good (0.61–0.80) or excellent (0.81–1.00). The agreement between two quantitative variables was also evaluated by the Bland–Altman plot analysis with 95% limits of agreement [274]. IQR/Median is a commonly used criterion in elastography [275]. However, on the old version of Affiniti software, which was used for a large part of the ElastPQ measurements performed for this study, there is no possibility to automatically calculate IQR/median, while mean and standard deviation (SD) are provided. To assess whether the use of quality criteria improves the diagnostic accuracy, the diagnostic performance was therefore evaluated overall and for the cases with ElastPQ SD/mean ≤30%).

F-TE was used as surrogate of histological fibrosis, and used as the reference method for the fibrosis staging adopting the cut-offs validated against liver biopsy in PSC (7.4, 8.6, 9.6 and 14.4 kPa for fibrosis stages ≥F1, ≥F2, ≥F3, and =F4, respectively) [14] and PBC (7.1, 8.8, 10.7 and 16.9 kPa for fibrosis stages ≥F1, ≥F2, ≥F3, and =F4, respectively) [13].

The diagnostic performance of ElastPQ for discrimination between staging categories was evaluated. ElastPQ cut-offs for each stage of fibrosis were established using receiver operating characteristics (ROC) curves to calculate sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV and NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR). The optimal cut-off values were chosen maximising the sum of sensitivity and specificity. Similarly, ROC curves were constructed to establish the performance (area under the curve (AUROC), Se, Sp, PPV,

NPV, PLR and NLR) of the elastographic techniques in predicting the presence of oesophageal varices. Optimal diagnostic cut-offs were calculated.

To identify the factors potentially affecting the concordance between the two elastographic techniques used to measure liver stiffness and to identify potential predictors of the presence of clinically significant portal hypertension, binary logistic regression analyses have been carried out. Potential multicollinearity between variables was checked by Spearman's rank correlation coefficient. All tests were two-sided and statistical significance was set at $P < 0.05$. Data analysis was performed with the SPSS 21 (IBM® SPSS®, Inc., Chicago, IL) and MedStat packages.

3.4 Ethics

The study was conducted in accordance with the Declaration of Helsinki and its latest amendments and approved by the local Ethics Committee (ref. NC01.14, 9190/ 20 March 2014). Signed informed consent was obtained from all the patients before taking part to the study.

4. Results

4.1 Non-invasive assessment of fibrosis stage in Primary Sclerosing Cholangitis

4.1.1 Baseline characteristics

One hundred fifty-two patients with PSC [93 males (61.2%), mean age 46 ± 16] were recruited in the study. The main characteristics of the studied population are described in **Tables 3** and **4**.

Measurements performed with both ElastPQ and F-TE were available for all patients, while spleen stiffness measurement was available in 109 (72%) patients.

Table 3. Clinical and biochemical characteristic of the patients affected by primary sclerosing cholangitis.

Number of patients	152
Age, years, mean ± SD	47 ± 16
Male sex, n (%)	93 (61.2)
Age at PSC diagnosis, years, mean ± SD	40 ± 16
PSC duration, months, median (IQR)	49 (97)
Large duct PSC/small duct PSC, n (%)	133/19 (87.5/12.5)
AIH overlap syndrome, n (%)	6 (3.9)
Disease localisation, n (%)	
Intra-hepatic	64 (43.2)
Extra-hepatic	1 (0.7)
Intra- and extra-hepatic	83 (56.1)

Hepato-biliary cancer, n (%)	9 (5.9)
HCC	2 (1.3)
CCa	5 (3.3)
GB carcinoma	1 (0.7)
Inflammatory bowel disease, n (%)	108 (70.6)
Ulcerative colitis	92 (60.5)
Crohn's disease	14 (9.2)
Indeterminate	2 (1.3)
Bowel malignancy, n (%)	9 (5.9)
Dysplasia (low grade)	1 (0.7)
DALM	3 (2)
Dysplasia (high grade)	1 (0.7)
Colon carcinoma	4 (2.6)
Cirrhosis at imaging, n (%)	45 (29.6)
History of ascites, n (%)	13 (8.6)
History of HE, n (%)	0 (0)
History of variceal bleeding, n (%)	3 (2)
OGD overall, n (%)	70 (45.7)
OGD within 12 months, n (%)	46 (30)
Oesophageal varices, n (%)	21 (13.7)
High risk oesophageal varices, n (%)*	3/46 (6.5)
Gastric varices, n (%)	2 (1.3)
Portal hypertensive gastropathy, n (%)	18 (11.7)
Child-Pugh class, n (%)	
A	131 (87.3)
B	19 (12.4)
C	0 (0)
MELD score, median (IQR)	7.5 (3)

PSC Mayo risk score, median (IQR)	-0.31 (1.35)
Biochemistry at the time of elastography	
Bilirubin (mg/dL), median (IQR)	0.7 (0.9)
Albumin (g/dL), median (IQR)	4.4 (0.6)
AST (IU/L), median (IQR)	45 (62)
ALT (IU/L), median (IQR)	53 (81)
ALP (IU/L), median (IQR)	165 (247)
PLTs, mean ± SD	250 (105)
Serum creatinine (mg/dl), mean ± SD	0.8 ± 0.2
Serum Na, mean ± SD	141 ± 2
INR, median (IQR)	1.0 (0.1)

** High-risk varices were defined as grade ≥2 oesophageal varices, or any varix with a red colour sign.*

Values are expressed as median (IQR), unless otherwise specified. Abbreviations: SD, standard deviation; PSC, primary sclerosing cholangitis; AIH, autoimmune hepatitis; HCC, hepatocellular carcinoma; CCa, cholangiocarcinoma; GB, gallbladder; DALM, dysplasia-associated lesion or mass; HE, hepatic encephalopathy; OGD, oesophagogastroduodenoscopy; MELD, model of end-stage liver disease; IQR, interquartile range; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; PLTs, platelets; Na, sodium; INR, International Normalized Ratio.

Table 4. Fibrosis scores, sonographic characteristics and elastography in the group of PSC patients.

Fibrosis scores at the time of elastography	
APRI index	0.01 (0.01)
AST/ALT ratio	0.91 (0.61)
FIB-4	1.17 (1.34)
Fibrosis index score, mean ± SD	1.26 ± 1.22
King's score	9.5 (12.8)
Lok score	-1.2 (1.9)
Fibro Q score	1.7 (2.1)
GUCI score	0.59 (0.84)
Bonacini score, mean ± SD	4 ± 2
ELF score*	10.14 ± 1.6
Liver stiffness measurement by F-TE	
Probe M/XL, n (%)	150/2 (99/1)
F-TE (median, kPa)	8.4 (14)
F-TE IQR (kPa)	1.1 (2.0)
F-TE IQR/median%	14 (10)
ElastPQ liver stiffness	
ElastPQ liver stiffness (average, kPa)	9.23 (13.2)
ElastPQ liver SD (kPa)	1.69 (2.7)
ElastPQ liver stiffness (median, kPa)	9.01 (12.69)
ElastPQ liver stiffness SD/average (%)	19 (11)
ElastPQ liver SD/average ≤30%	130 (85.5)

ElastPQ spleen stiffness	
ElastPQ spleen failure, n (%)	19 (14.7)
ElastPQ spleen stiffness (average, kPa)	29.65 (16.10)
ElastPQ spleen stiffness SD (kPa)	6.03 (4.51)
ElastPQ spleen stiffness (median, kPa)	28.78 (14.52)
ElastPQ spleen stiffness SD/average (%)	22 (13)
Fibrosis Stage assessed with F-TE as reference standard	
F0	67 (44.1)
F1	10 (6.6)
F2	9 (5.9)
F3	19 (12.5)
F4	47 (30.9)
Spleen size, cm, mean ± SD	11.9 ± 3.4
Spleen area, cm2**	40 (28.5)
LSPS	0.47 (1.05)
SSPSA	5.20 (9.06)
Baveno VI criteria	
Low risk, n (%)	105 (70)
High risk, n (%)	45 (30)
Expanded Baveno VI criteria	
Low risk, n (%)	116 (77.3)
High risk, n (%)	34 (22.7)

** ELF score at the time of elastography was available in 43 (28.3%) patients.*

*** Spleen area was available for 111 (73%) patients.*

Values are expressed as median (IQR), unless otherwise specified. Abbreviations: APRI, AST to platelet ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase; SD, standard deviation; ELF, enhanced liver fibrosis; F-TE, transient elastography performed by FibroScan.

4.1.2 Comparison between shear wave elastography (ElastPQ) and F-TE.

ElastPQ liver stiffness demonstrated to have an excellent correlation with F-TE regardless of whether ElastPQ mean value (P<0.0001, Spearman's correlation coefficient 0.93; Lin's correlation coefficient 0.87, 95%CI 0.83-0.91) or ElastPQ median value was considered (P<0.0001, Spearman's correlation coefficient 0.93; Lin's correlation coefficient 0.86, 95%CI 0.82-0.90) (**Figure 18 A** and **B**). The comparison of the correlation's coefficients for mean ElastPQ and median ElastPQ liver stiffness did not show a statistically significant difference (P=0.596).

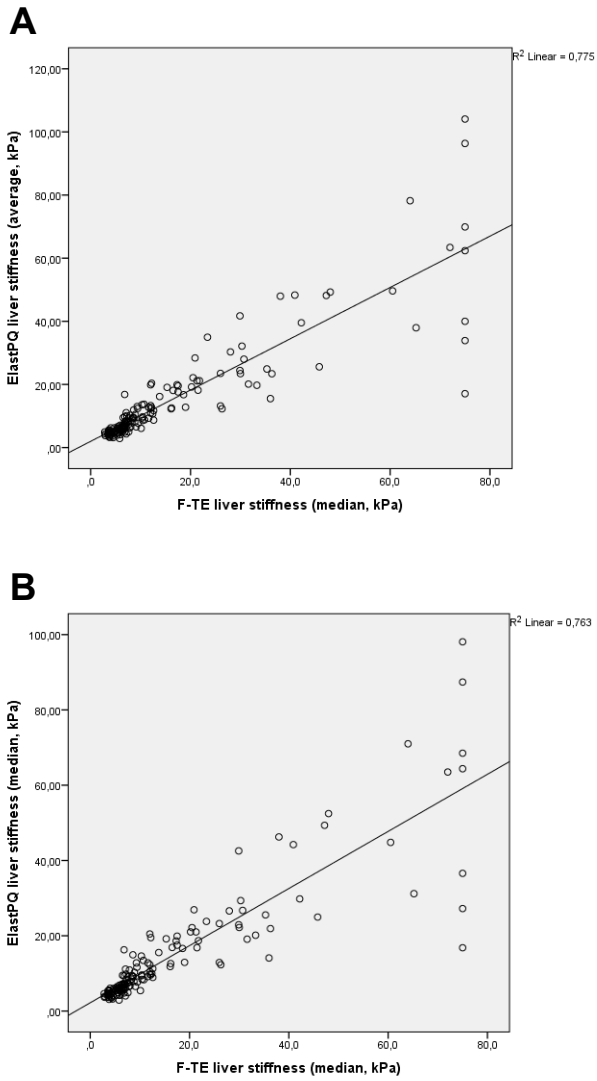


Figure 18. *Linear correlation between F-TE and ElastPQ mean (**A**) or median (**B**) in the overall PSC population.*

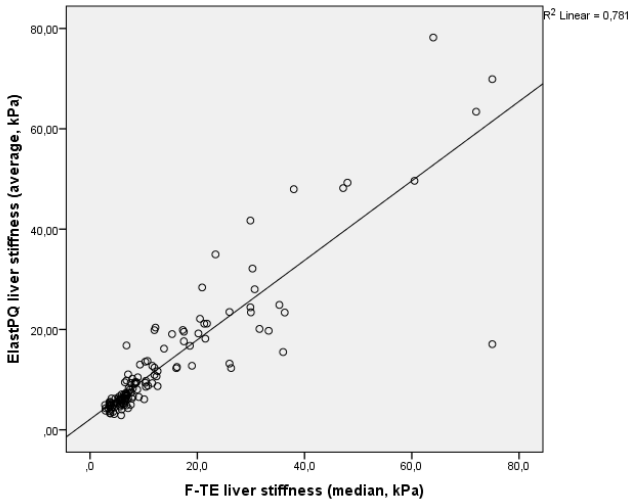
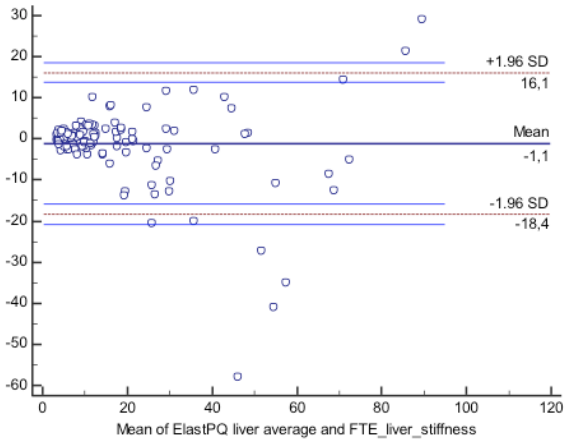


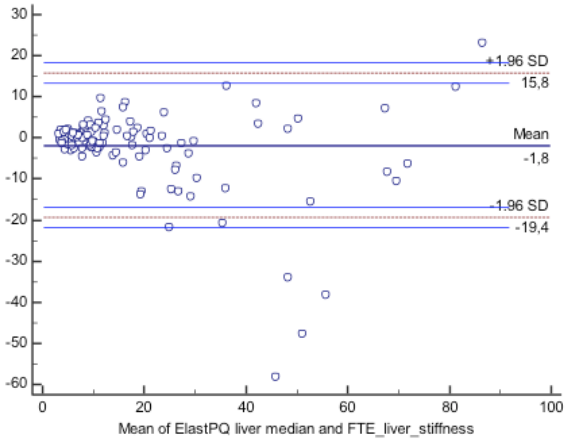
Figure 19. *Linear correlation between F-TE and ElastPQ in the subgroup with ElastPQ SD/mean ≤30%.*

The application of quality criterion (ElastPQ SD/mean ≤30%), which was met by 130 patients (86%), did not influence the test accuracy (P<0.0001, Spearman's correlation coefficient 0.92; Lin's correlation coefficient 0.88, 95%CI 0.83 to 0.91 (**Figure 19**). Bland-Altman plot agreement analyses are shown in the figures below (**Figures 20 A, B** and **C**).



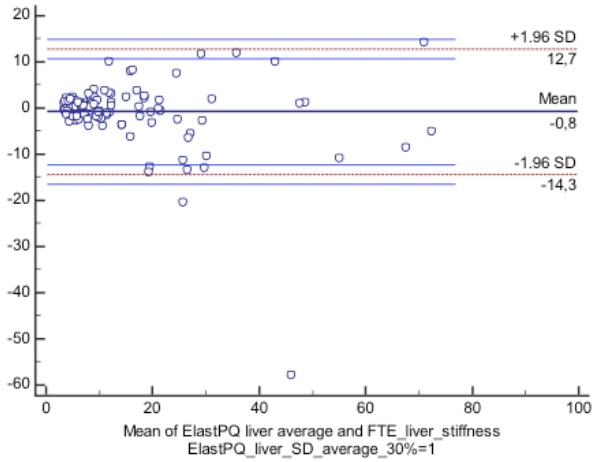
Sample size	152
Arithmetic mean	-1,1273
95% CI	-2,5369 to 0,2823
P (H_0: Mean=0)	0,1162
Standard deviation	8,7958
Lower limit	-18,3670
95% CI	-20,7813 to -15,9527
Upper limit	16,1124
95% CI	13,6981 to 18,5267

Figure 20 A. *Bland-Altman plot agreement analysis between F-TE and mean ElastPQ in the overall PSC population.*



Sample size	152
Arithmetic mean	-1,7920
95% CI	-3,2313 to -0,3528
P (H_0: Mean=0)	0,0150
Standard deviation	8,9806
Lower limit	-19,3940
95% CI	-21,8590 to -16,9290
Upper limit	15,8099
95% CI	13,3449 to 18,2750

Figure 20 B. *Bland-Altman plot agreement analysis between F-TE and median ElastPQ in the overall PSC population.*



Sample size	130
Arithmetic mean	-0,8124
95% CI	-2,0108 to 0,3860
P (H_0: Mean=0)	0,1822
Standard deviation	6,9061
Lower limit	-14,3483
95% CI	-16,4016 to -12,2950
Upper limit	12,7235
95% CI	10,6702 to 14,7768

Figure 20 C. *Bland-Altman plot agreement analysis between F-TE and mean ElastPQ in the subgroup with ElastPQ SD/mean ≤30%.*

The continuous line is the mean of the differences. The dashed grey lines define the 95% limits of agreement, with the blue lines representing the confidence interval (CI) (mean of the difference (±2SD)).

4.1.3 Correlation between liver elastography and surrogate markers of liver fibrosis.

Liver ElastPQ, as well as F-TE, showed a significant correlation with all the commonly used non-invasive scores of fibrosis (P<0.001), with the exception for AST/ALT ratio (**Table 5**). The best Spearman's coefficient (rho) was found for the APRI index (0.72 with regards to F-TE, 0.69 with regards to ElastPQ) and with the ELF score (0.71 with regards to F-TE, 0.64 with regards to ElastPQ). The correlation with the ELF score was clearly limited by the small number of patients with an available ELF test at the time of elastography.

Table 5. Correlation between liver elastography and surrogate markers of liver fibrosis.

		APRI	AST/ALT	FIB-4	King's score	Fibrosis index	Lok score	Fibro Q	GUCI	Bonacini	ELF
F-TE	*r*	0.721	0.132	0.488	0.664	0.391	0.303	0.208	0.732	0.267	0.714
	P	<0.000	0.107	<0.000	<0.000	<0.000	<0.000	0.011	<0.000	0.001	<0.000
ElastPQ liver (mean)	*r*	0.693	0.085	0.475	0.641	0.398	0.280	0.199	0.704	0.236	0.643
	P	<0.000	0.339	<0.000	<0.000	<0.000	0.001	0.025	<0.000	0.008	<0.000
ElastPQ liver (median)	*r*	0.690	0.080	0.474	0.642	0.385	0.269	0.196	0.703	0.228	0.639
	P	<0.000	0.367	<0.000	<0.000	<0.000	0.002	0.027	<0.000	0.010	<0.000

Abbreviations: APRI, AST to Platelet Ratio Index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GUCI, Göteborg University Cirrhosis Index; ELF, enhanced liver fibrosis (score).

4.1.4. Correlation between elastography, prognostic scores and serum markers of inflammation and cholestasis.

The correlation between liver elastography (measured both with FibroScan and ElastPQ), spleen elastography and the currently validated prognostic scores in PSC were also assessed. Elastography of both liver and spleen was significantly correlated with the MELD, the PSC Mayo risk score and the Oxford-Amsterdam score **(Table 6)**. However, the highest correlation coefficient were found for the Mayo risk score, both for F-TE (Spearman's rho 0.65, P<0.001) and liver ElastPQ (Spearman's rho 0.64, P=0.002). Still statistically significant but much weaker correlations with all the prognostic scores were found for ElastPQ spleen stiffness (Spearman's rho 0.26 (P=0.007), 0.38 (P<0.001) and 0.44 (P<0.001) for MELD, PSC Mayo risk score and Oxford-Amsterdam score, respectively).

As previously reported [14], liver stiffness measured by F-TE was positively correlated with serum total bilirubin, alkaline phosphatase (ALP), and transaminases levels (P<0.001). This significant correlation was maintained, with similar correlation coefficients, when liver stiffness measurement was performed with ElastPQ. With regards to ElastPQ spleen stiffness, instead, the correlation with the above-mentioned markers of inflammation and cholestasis was poor (**Table 6**).

Table 6. Correlation between liver elastography, prognostic scores and serum markers of inflammation and cholestasis.

		MELD	PSC Mayo RS	Oxford-Amsterdam score	ALT	AST	Bilirubin	ALP
F-TE	*r*	0.392	0.649	0.568	0.494	0.655	0.587	0.476
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ElastPQ liver (mean)	*r*	0.450	0.643	0.577	0.462	0.637	0.601	0.461
	P	0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001
ElastPQ liver (median)	*r*	0.455	0.651	0.574	0.470	0.643	0.608	0.468
	P	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001
ElastPQ spleen (mean)	*r*	0.259	0.377	0.442	0.171	0.320	0.224	0.232
	P	0.007	<0.001	<0.001	0.075	0.001	0.019	0.015
ElastPQ spleen (median)	*r*	0.295	0.364	0.451	0.193	0.328	0.237	0.228
	P	0.002	0.000	<0.001	0.044	0.000	0.013	0.017

Abbreviations: MELD, model of end-stage liver disease; PSC, primary sclerosing cholangitis; RS, risk score; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

4.1.5 Performance of liver ElastPQ in differentiating liver fibrosis stages.

With a few exceptions, histology is no longer considered necessary for establishing a diagnosis of PSC in patients with characteristic appearance on cholangiogram [1, 77] and therefore is not routinely performed in these patients. Accordingly, no recent liver biopsy was available for most of the patients included in this cohort. In order to establish the optimal cut-off of liver stiffness measured by ElastPQ for each grade of fibrosis, F-TE was used as a surrogate of histological fibrosis, adopting the

cut-offs recently validated against liver biopsy in PSC [14]. AUROC curves were obtained for each stage of fibrosis (**Figures 22-25**). AUROCs (95%CI) for ElastPQ were 0.96 (0.91–0.98), 0.97 (0.93–0.99), 0.97 (0.93–0.99), 0.99 (0.95–0.99) for fibrosis stage F≥1, F≥2, F≥3 and F=4 respectively. Optimal cut-off values were 7.4 kPa (91% sensitivity, 92% specificity), 8.5 kPa (95% sensitivity, 88% specificity), 10.5 kPa (89% sensitivity, 94% specificity) and 12.1 kPa (100% sensitivity, 89% specificity) for mild, moderate, severe fibrosis and cirrhosis, respectively (**Table 7**). The results remained similar when patients with AIH overlap where excluded from the analysis. The distribution of liver ElastPQ values according to fibrosis stage defined by F-TE is shown in **Figure 21**.

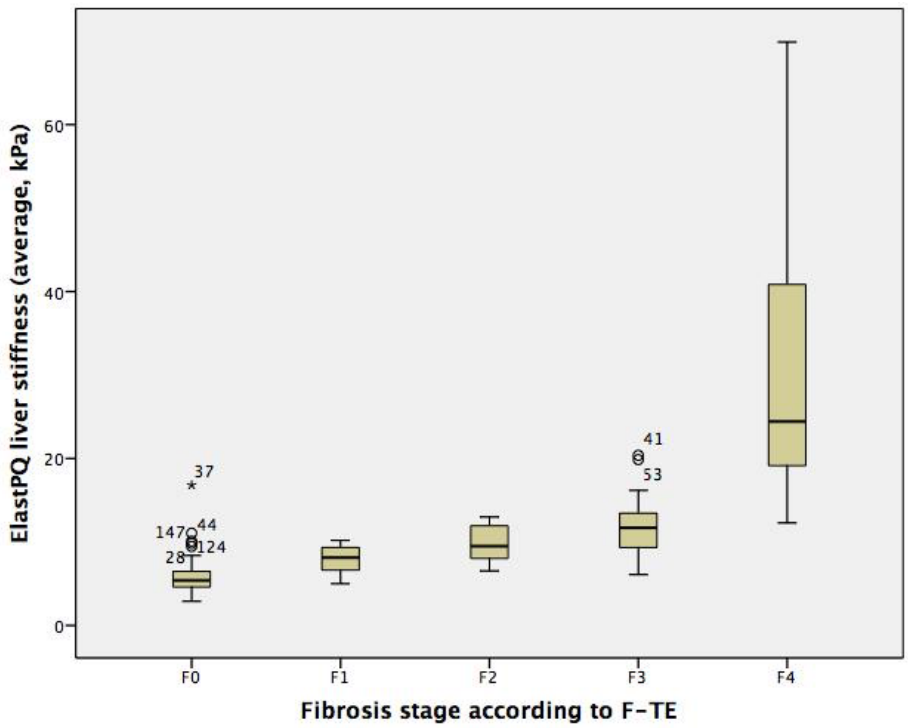


Figure 21. *Distribution of ElastPQ liver stiffness values according to fibrosis stage as defined by F-TE.*

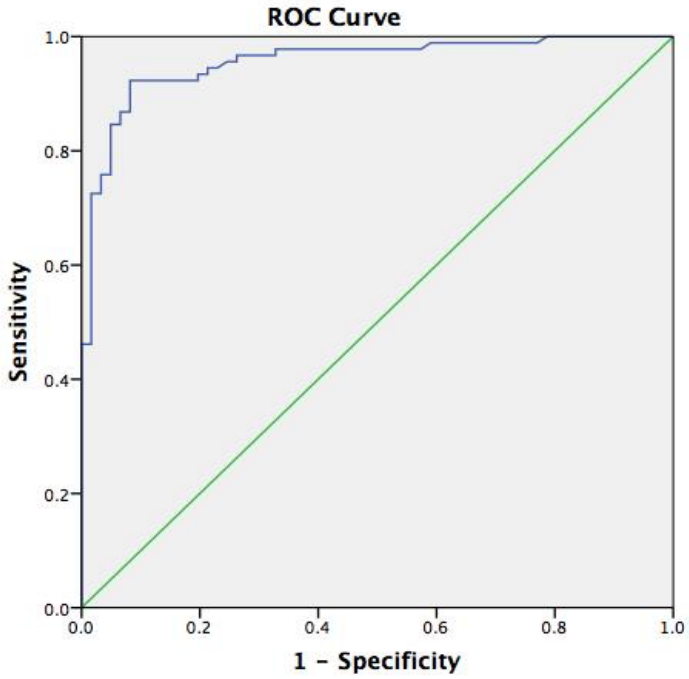


Figure 22. *Receiver operating characteristic analysis of the ability of liver ElastPQ to predict mild fibrosis (F≥1) in PSC.*

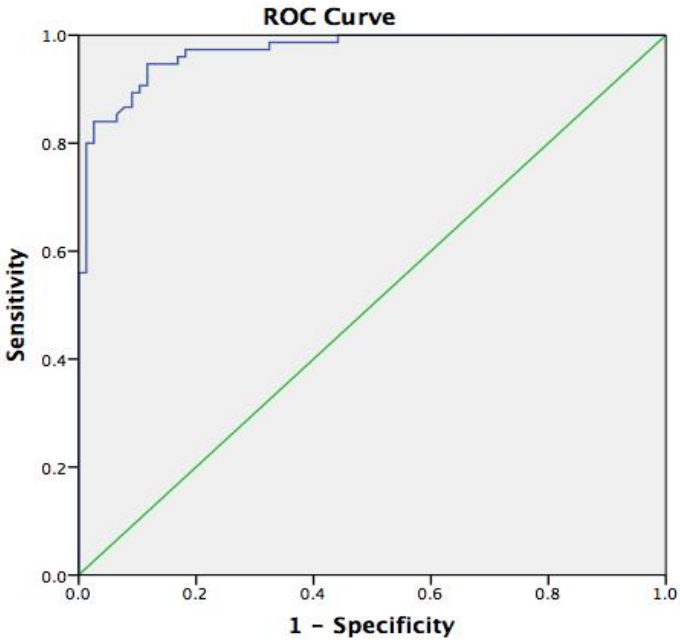


Figure 23. *Receiver operating characteristic analysis of the ability of liver ElastPQ to predict moderate fibrosis (F≥2) in PSC.*

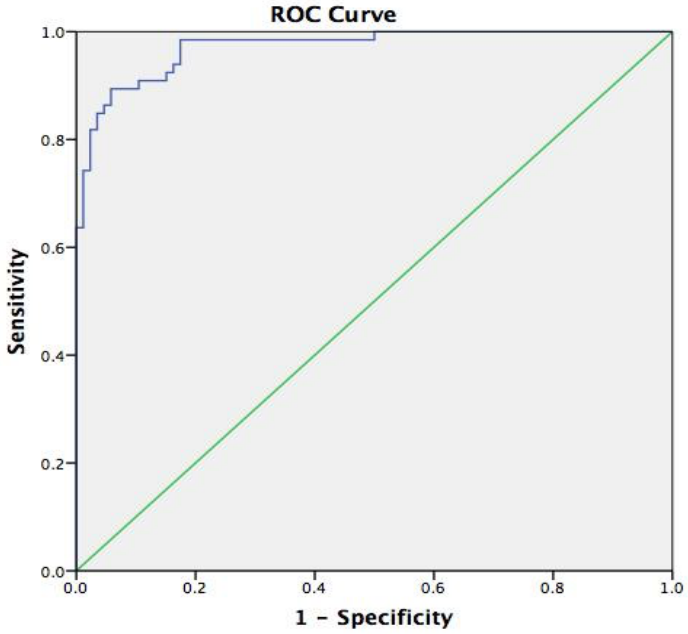


Figure 24. *Receiver operating characteristic analysis of the ability of liver ElastPQ to predict severe fibrosis (F≥3) in PSC.*

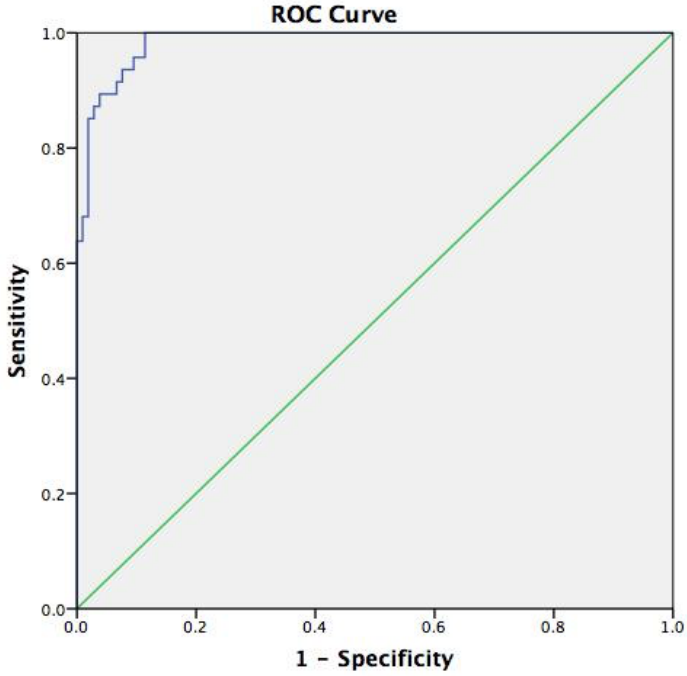


Figure 25. *Receiver operating characteristic analysis of the ability of liver ElastPQ to predict cirrhosis (F=4) in PSC.*

Table 7. Performance of ElastPQ in differentiating liver fibrosis stages (according to F-TE) in PSC.

Stage	N.	Cut-off (kPa)	Se	Sp	PPV	NPV	PLR	NLR	AUROC (95%CI)
≥ F1	91	7.4	0.91	0.92	0.94	0.88	11.4	0.10	0.96 (0.91-0.98)
≥ F2	75	8.5	0.95	0.88	0.89	0.94	7.9	0.06	0.97 (0.93-0.99)
≥ F3	66	10.5	0.89	0.94	0.92	0.92	14.8	0.12	0.97 (0.93-0.99)
F4	47	12.1	1.0	0.89	0.80	1.0	9.1	0	0.99 (0.95-0.99)

Abbreviations: kPa, kilopascal; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; AUROC, area under receiver operating characteristic curve; CI, confidence interval.

The application of the quality criterion ElastPQ SD/mean ≤30% did not improve significantly the performance of liver ElastPQ in discriminating liver fibrosis stages **(Table 8).**

Table 8. Performance of ElastPQ in differentiating liver fibrosis stages (according to F-TE) in PSC when the quality criterion ElastPQ SD/mean ≤30% was applied.

Stage	N.	Cut-off (kPa)	Se	Sp	PPV	NPV	PLR	NLR	AUROC (95%CI)	P*
≥ F1	74	7.4	0.89	0.95	0.96	0.87	17.8	0.12	0.949 (0.911-0.987)	*ns*
≥ F2	59	8.5	0.95	0.89	0.88	0.96	8.6	0.06	0.968 (0.942-0.944)	*ns*
≥ F3	53	10.5	0.87	0.96	0.94	0.91	21.8	0.14	0.969 (0.943-0.995)	*ns*
F4	36	12.1	1	0.90	0.80	1	10	0	0.982 (0.965-0.999)	*ns*

Abbreviations: kPa, kilopascal; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; AUROC, area under receiver operating characteristic curve; CI, confidence interval.

**P value refers to the comparison between the AUROC obtained for the overall population and the AUROC obtained when the quality criterion ElastPQ SD/mean ≤30% was applied.*

Table 9 reports the misclassification in staging liver fibrosis (defined by F-TE cut-offs), using the cut-offs obtained for ElastPQ liver stiffness.

Table 9. Misclassification in staging liver fibrosis using ElastPQ liver stiffness.

Fibrosis stage (F-TE)	FN	FP	TOT
ElastPQ Mean (Overall)			
F≥1	8/91 (9%)	5/61 (8%)	17%
F≥2	4/75 (5%)	9/77 (12%)	17%
F≥3	7/66 (11%)	5/86 (6%)	17%
F=4	0/47 (0%)	12/105 (11%)	11%
Total misclassified	19/152 (13%)	31/152 (20%)	33%
ElastPQ Mean (SD/mean ≤30%)			
F≥1	8/74 (11%)	3/56 (5%)	16%
F≥2	3/59 (5%)	8/71 (11%)	16%
F≥3	7/53 (13%)	3/77 (4%)	17%
F=4	0/36 (0%)	9/95 (10%)	10%
Total misclassified	18/130 (14%)	23/130 (18%)	32%

Abbreviations: F-TE, liver transient elastography performed with FibroScan; FN, false negative; FP, false positive; TOT, total; SD, standard deviation.

4.1.6 Factors influencing the discrepancy between F-TE and ElastPQ.

The factors potentially influencing the discrepancy between F-TE and ElastPQ values were examined. When a difference between the two techniques of ± 2 kPa was considered, statistically significant variables at the univariate analysis were the Mayo risk score, total bilirubin, ALT, AST, ALP, PLTs and the presence of cirrhosis (**Table 11**). On multivariate, only the diagnosis of cirrhosis (either defined by F-TE values or diagnosed at imaging) maintained its statistical significance (OR

9.87, 95%CI 3.35-29.10, P<0.001). The results did not change using a delta liver stiffness of 5 or 10 kPa (data not shown).

Table 10. Factors associated with a difference of ± 2 kPa between liver stiffness measured by F-TE and ElastPQ in the PSC cohort.

	Univariate			Multivariate		
	F-TE - ElastPQ liver < 2 kPa	F-TE - ElastPQ liver ≥2 kPa	P	OR	95% CI	P
PSC Mayo RS	-0.46 (1.22)	0.34 (1.49)	<0.000			
MELD score	7 (3)	7 (4)	0.611			
AIH overlap	3/119 (2.5)	3/33 (9.1)	0.117			
Small duct PSC	17/119 (14.3)	2/33 (6.1)	0.251			
Age, years	46 ± 16	49 ± 16	0.501			
Bilirubin (mg/dL)	0.58 (1.0)	0.94 (0.8)	0.009			
ALT (IU/L)	45 (73)	78 (68)	<0.000			
AST (IU/L)	37 (55)	67 (60)	<0.000			
ALP (IU/L)	145 (256)	222 (193)	0.147			
Platelets/mmc	255 (129)	200 (142)	0.023			
Cirrhosis (F-TE = F4)	22/119 (18)	25/33 (76)	<0.000	*9.87*	*3.38-28.88*	*<0.000*

Numerical variables are expressed as median (IQR) or mean ± SD, dichotomous variables are expressed as n (%).

Abbreviations: F-TE, liver transient elastography performed with FibroScan; kPa, kilopascal; OR, odd ratio; CI, confidence interval; PSC, primary sclerosing cholangitis; RS, risk score; MELD, model of end-stage liver disease; AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase, IQR, interquartile range; SD, standard deviation.

4.2 Non-invasive assessment of portal hypertension in PSC.

The factors potentially associated with the presence of clinically significant portal hypertension (CSPH), therefore with the presence of oesophageal varices (OV), were investigated. This analysis was performed only in the subgroup of 46 patients who underwent an upper-GI endoscopy within 12 months from the date of the elastographic assessment. Eighteen patients had OVs in this group, with only two patients having varices at high-risk of bleeding (HRVs). The differences in clinical, biochemical and elastographic parameters between the groups of patients with and without oesophageal varices are shown in **Table 11**. In particular, median values of F-TE, liver ElastPQ, spleen stiffness and LSPS were significantly higher in patients with OV compared to those without OV (**Figures 26-29**). On univariate analysis, a statistically significant association of the presence of oesophageal varices was found with serological parameters such as bilirubin, platelet count, albumin and INR, spleen longitudinal diameter (but not with spleen area), liver stiffness measured both with F-TE and ElastPQ, spleen stiffness, the composite scores LSPS, Child-Pugh, MELD and Mayo risk score (**Table 12**). Different models of multivariate logistic regression analysis were performed including the variables statistically significant in the univariate analysis. ElastPQ spleen stiffness was the only independent predictor of CSPH (OR 1.137; CI 1.02-1.27; p <0.021) (**Table 12**), regardless of whether the Mayo risk score, MELD or Child-Pugh score was included in the model as indicator of liver disease severity. Similarly, the results did not change when either F-TE or ElastPQ were included in the multivariate analysis as measure of liver stiffness. Finally, spleen stiffness remained the only variable statistically significant in the multivariate analysis model including the LPSP score (OR 1.124; CI 1.01-1.26; p <0.034) (**Table 12**).

Table 11. Comparison between patients with and patients without oesophageal varices.

	No OVs	OVs	P
PSC Mayo RS	-0.27 (1.43)	1.06 (1.5)	<0.000
Child-Pugh score	5.0 (0)	7 (3)	<0.000
MELD score	7.01 (3)	12.46 (7)	<0.000
Spleen area, cm2	55 (56)	87 (55)	0.040
Spleen LD, cm	12 (4.4)	16 (4.3)	0.007
Bilirubin mg/dL	0.9 (1.2)	3.0 (5.5)	<0.000
AST (IU/L)	70 (60)	81 (71)	0.045
ALP (IU/L)	256 (292)	210 (243)	0.566
Platelets/mmc	254 (483)	102 (138)	0.001
Albumin g/dL	4.3 (0.6)	3.4 (0.9)	0.001
INR	1 (0.1)	1.2 (0.8)	<0.000
F-TE liver stiffness, kPa	12 (22.2)	46.5 (44.5)	<0.000
ElastPQ liver stiffness, kPa	12 (14.1)	39.8 (28)	<0.000
ElastPQ spleen stiffness, kPa	31.6 (14.1)	51.8 (26.5)	<0.000
LSPS	0.61 (2.1)	6.3 (12.6)	<0.000
SSPSA	11.6 (18.2)	28.9 (41.9)	0.020
ELF score*	11.4 (0.9)	13.4 (1.6)	0.006

Values are expressed as median (IQR) or mean ± SD, according to distribution.
**ELF available only in 11 patients with an OGD within 1 year.*
Abbreviations: OVs, oesophageal varices; PSC, primary sclerosing cholangitis; RS, risk score; MELD, model of end-stage liver disease; LD, longitudinal diameter; INR, international normalized ratio; F-TE, liver transient elastography performed with FibroScan; kPa, kilopascal; ELF, enhanced liver fibrosis score.

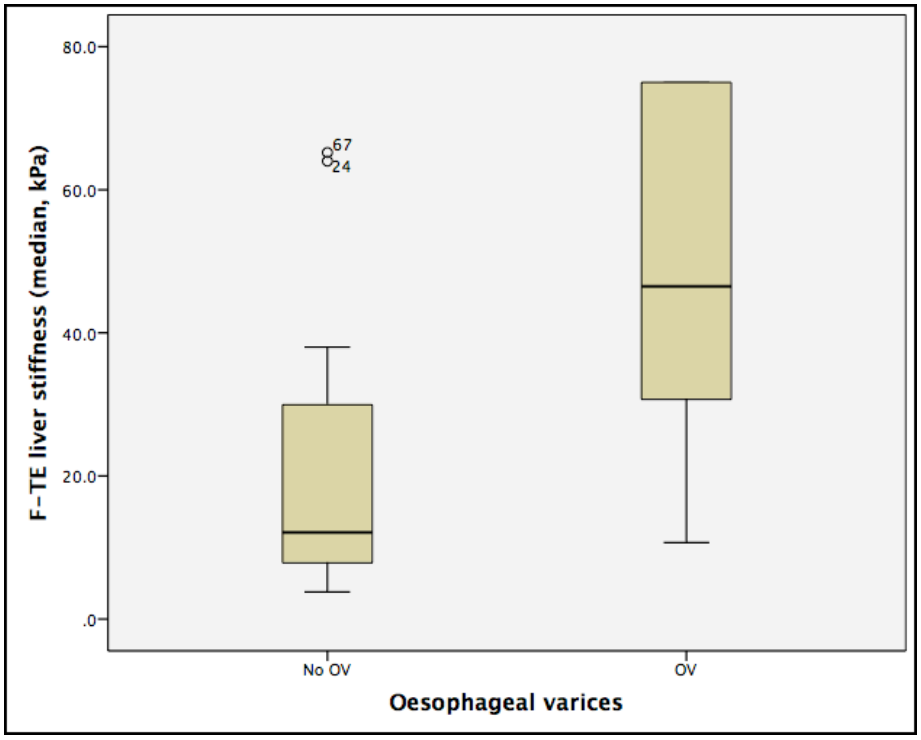


Figure 26. *Difference in the median values of F-TE between patients with and without oesophageal varices (P<0.000).*

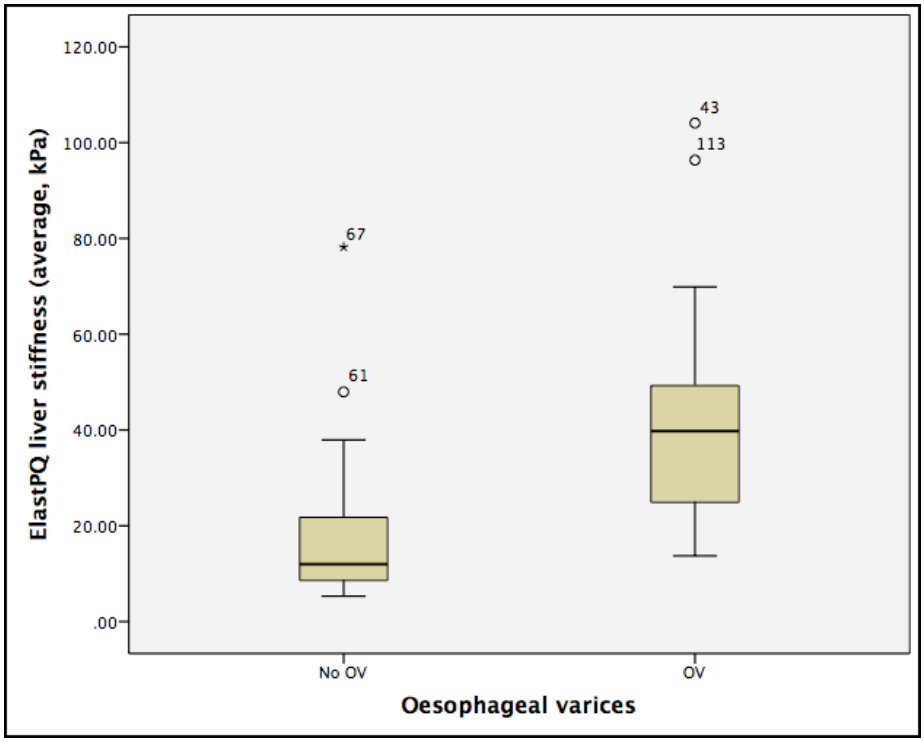


Figure 27. *Difference in the median values of ElastPQ liver stiffness between patients with and without oesophageal varices (P<0.000).*

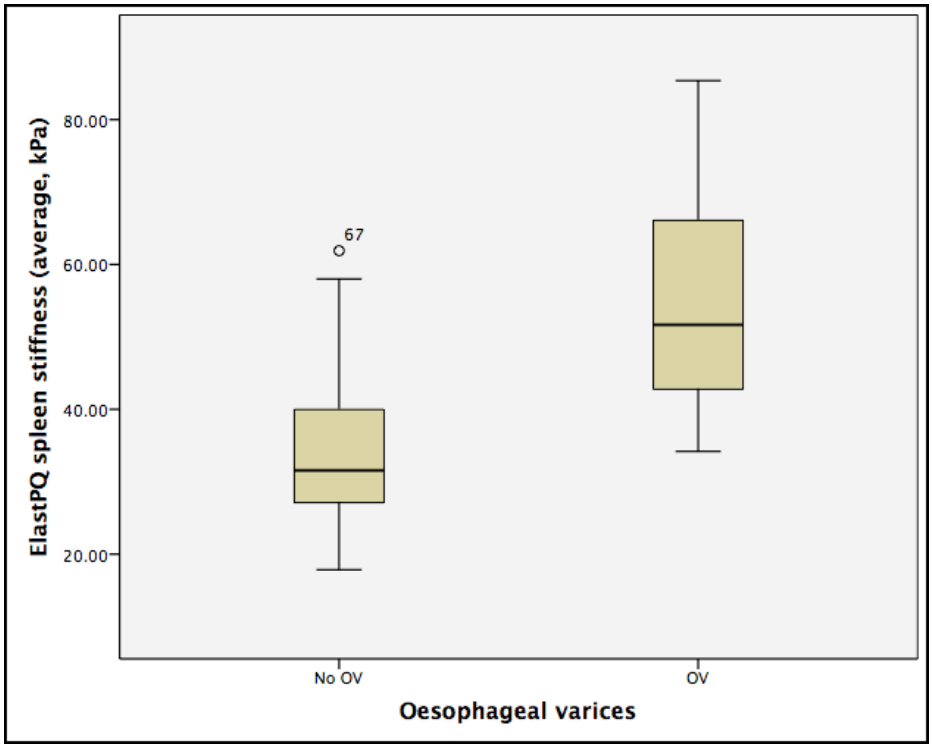


Figure 28. *Difference in the median values of ElastPQ spleen stiffness between patients with and without oesophageal varices (P<0.000).*

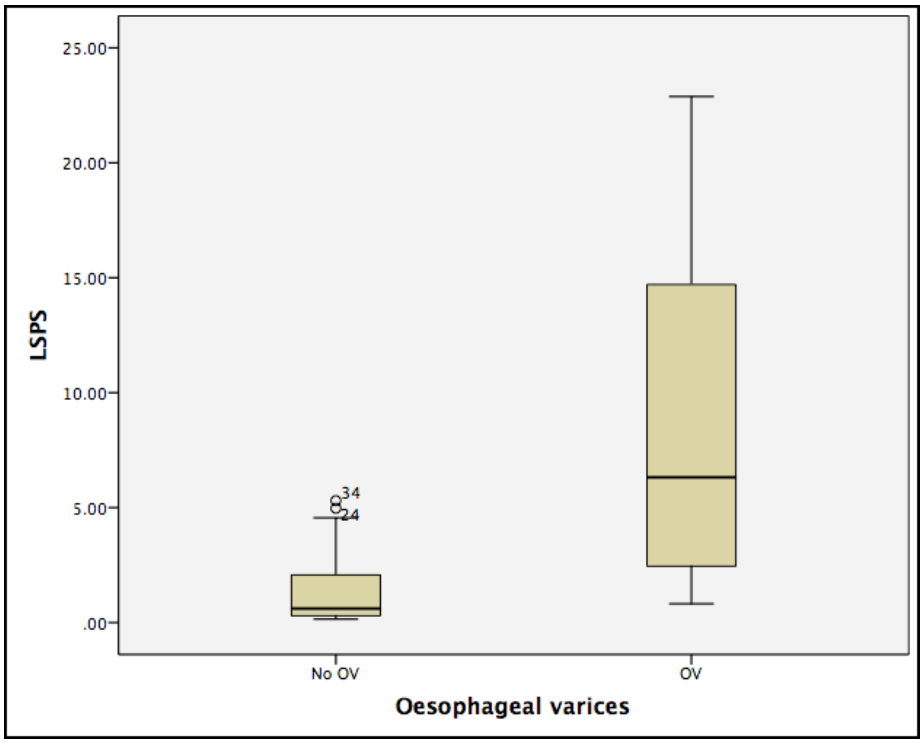


Figure 29. *Difference in the median values of LSPS score between patients with and without oesophageal varices (P<0.000).*

Table 12. Univariate and multivariate logistic regression analysis of factors potentially associated with the presence of clinically significant portal hypertension in PSC patients with an OGD within 12 months.

	Univariate			Multivariate		
	OR	**95% CI**	**P**	**OR**	**95% CI**	**P**
PSC Mayo RS	3.672	1.694-7.962	0.001			
Child-Pugh score	3.868	1.780-8.402	0.001			
MELD score	1.634	1.215-2.198	0.001			
Spleen area, cm^2	1.029	1.000-1.059	0.054			
Spleen LD, cm	1.264	1.040-1.535	0.018			
Blirubin mg/dL	1.901	1.185-3.048	0.008			
AST (IU/L)	1.012	0.999-1.025	0.063			
ALP (IU/L)	0.999	0.996-1.001	0.412			
Platelets/mmc	0.994	0.988-1.000	0.043			
Albumin g/dL	0.090	0.020-0.397	0.001			
INR	45619	62-3347…	0.001			
F-TE liver stiffness, kPa	1.075	1.032-1.121	0.001			
ElastPQ liver stiffness, kPa	1.073	1.024-1.124	0.003			
ElastPQ spleen stiffness, kPa	1.123	1.037-1.216	0.004	*1.137*	*1.020-1.268*	*0.021*
LSPS	1.741	1.199-2.527	0.004			
SSPSA	1.056	0.987-1.130	0.112			
ELF score*	3.293	NA	0.996			

**ELF score was available for only 11 patients in this group.*

Abbreviations: OR, odd ratio; CI, confidence interval; PSC, primary sclerosing cholangitis; RS, risk score; MELD, model of end-stage liver disease; LD, longitudinal diameter; AST, aspartate aminotransferase; ALP, alkaline phosphatase; INR, international normalized ratio; F-TE, liver elastography performed with FibroScan; kPa, kilopascal; ELF, enhanced liver fibrosis score.

The ability of F-TE, ElastPQ liver stiffness, and ElastPQ spleen stiffness in predicting the presence of oesophageal varices was evaluated and compared with the performance of the other non-invasive markers of liver fibrosis. ROC curves were obtained for each non-invasive test (**Tables 13** and **14**). The performance of ELF score could not be evaluated in this cohort owing to the very small number of patients with available ELF test (n.11). F-TE, liver ElastPQ and spleen ElastPQ performed better than the other non-invasive diagnostic tests, showing similar ROC curves. AUROCs (95%CI) were: 0.88 (0.78-0.98) for F-TE, 0.87 (0.77-0.97) for ElastPQ liver stiffness and 0.87 (0.76-0.99) for ElastPQ spleen stiffness. Also LSPS was found to have a fairly high predictive value in detecting the presence of OVs (AUROC 0.90, 95%CI 0.81-0.99) (**Table 13**, **Figure 30**).

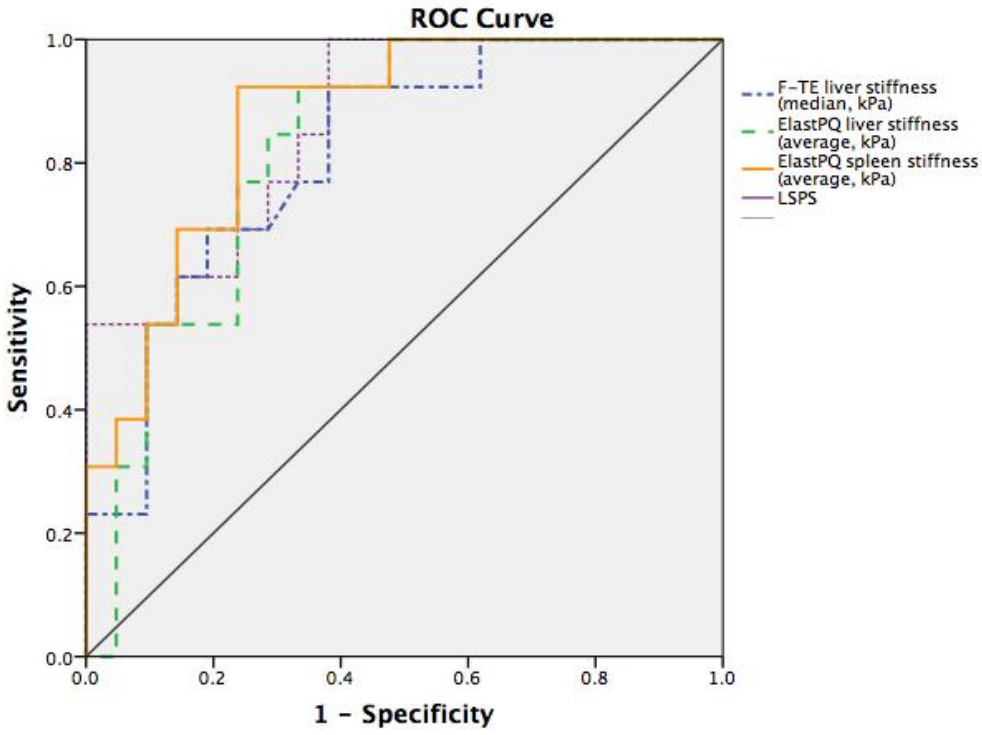


Figure 30. *Receiver operating characteristic analysis of the ability of F-TE, ElastPQ liver stiffness, ElastPQ spleen stiffness and LSPS to predict the presence of oesophageal varices in PSC.*

The best cut-off value for predicting the presence of OVs was then calculated for each curve, and the diagnostic accuracy was analysed. F-TE, ElastPQ liver and spleen stiffness demonstrated a good sensitivity, however specificity was relatively low, ranging between 77% and 68%. Accordingly, while NPVs and NLRs were good, PPV and PLR were modest for all tests (**Table 13**). No statistically significant difference was detected between the AUROCs of F-TE, ElastPQ liver stiffness and ElastPQ spleen stiffness (P value not significant for all the comparisons), indicating a similar performance of the three techniques, as well as the LSPS. The AUROC of ElastPQ liver stiffness did not improve significantly when the quality criterion SD/mean ≤30% was applied (data not shown).

Table 13. Performance of liver stiffness (F-TE and ElastPQ), ElastPQ spleen stiffness and LSPS in predicting the presence of oesophageal varices in PSC.

Test	OVs/n	Se	Sp	PPV	NPV	PLR	NLR	Cut-off value (kPa)	AUROC (95% CI)
F-TE liver	18/46	0.94	0.68	0.65	0.95	2.9	0.09	18.5	0.880 (0.783-0.977)
ElastPQ liver	18/46	0.89	0.75	0.70	0.91	3.6	0.15	21.1	0.871 (0.770-0.972)
ElastPQ spleen	13/35	0.92	0.77	0.71	0.94	4	0.10	40.2	0.874 (0.760-0.988)
LSPS	18/46	0.83	0.74	-	-	-	-	-	0.899 (0.812-0.986)

Abbreviations: PSC primary sclerosing cholangitis; F-TE, transient elastography performed with FibroScan; LSPS, liver stiffness X spleen diameter/platelet count; OVs, oesophageal varices; n, number of patients; Se, sensitivity; SP, specificity; PPV, positive predictive value; NPV negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; AUROC, area under receiver operating characteristic curve; CI, confidence interval.

All the other non-invasive diagnostic tests had smaller AUROCs, with the exception of the Lok score, which reached an area of 0.87 (95%CI 0.74-1.00) (**Table 14**), when evaluated in the overall cohort of 46 patients with OGD within 12 months. However, when the AUROCs were performed in the sub-population of the patients who had spleen stiffness measurement (n. 35, 13 with OVs), ElastPQ spleen stiffness showed the best AUROC (0.87, 95%CI 0.75-0.98), followed by the LSPS (AUROC 0.86, 95%CI 0.74-0.98) and then by the Lok score (AUROC 0.85, 95%CI 0.68-1.00) (**Figure 31**).

Table 14. Performance of the non-invasive scores of liver fibrosis in predicting the presence of oesophageal varices in PSC.

Test	OVs/n	AUROC (95% CI)
APRI	18/46	0.813 (0.686-0.941)
AST/ALT	18/46	0.819 (0.689-0.950)
FIB-4	18/46	0.812 (0.667-0.954)
King's score	18/46	0.815 (0.678-0.952)
Fibrosis Index	18/46	0.839 (0.691-0.988)
Lok score	18/46	0.873 (0.744-1.000)
Fibro Q	18/46	0.798 (0.655-0.941)
GUCI	18/46	0.853 (0.736-0.970)
Bonacini score	18/46	0.851 (0.727-0.975)

Abbreviations: APRI, aspartate aminotransferase-to- platelet ratio index, AST to Platelet Ratio Index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GUCI, Göteborg University Cirrhosis Index.

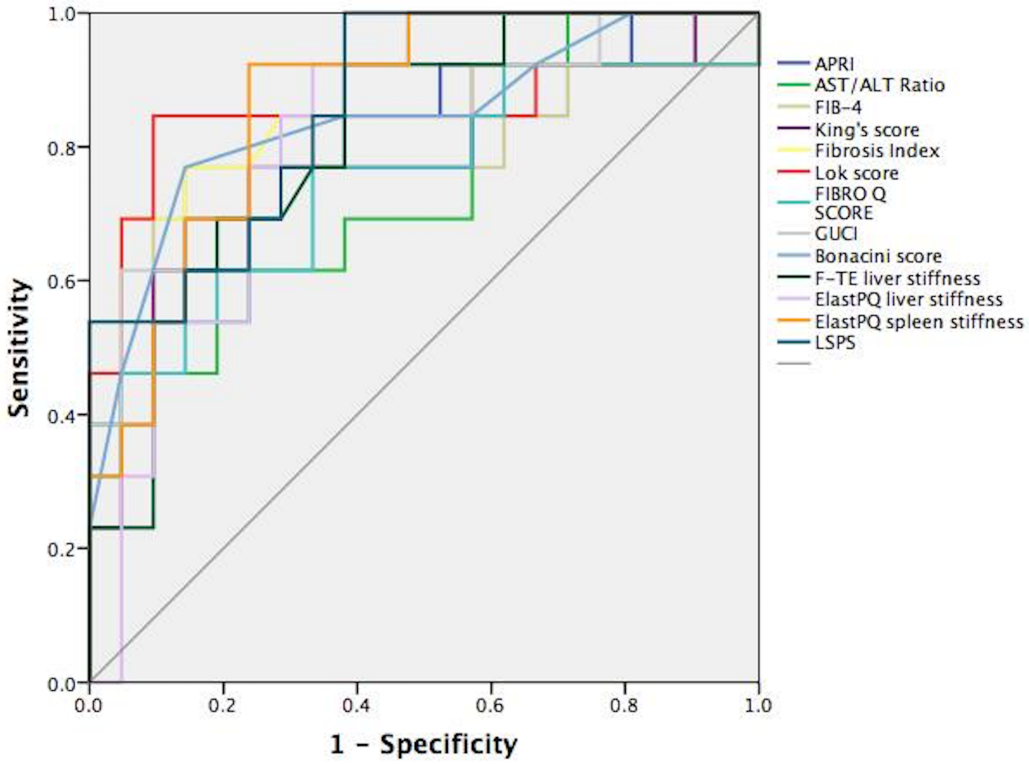


Figure 31. *Receiver operating characteristic analysis of the ability of the non-invasive scores of liver fibrosis, liver and spleen elastography to predict the presence of oesophageal varices.*

Abbreviations: APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GUCI, Göteborg University Cirrhosis Index; F-TE, transient elastography performed with FibroScan; LSPS, liver stiffness X spleen diameter/platelet count.

Comparison of ElastPQ spleen stiffness with the Baveno VI criteria.

Finally, the performance of ElastPQ spleen stiffness in predicting CSPH was evaluated in comparison with the currently validated Baveno VI and expanded Baveno VI criteria [228, 233]. This analysis was performed only on 35 patients who had an OGD within a year from the elastographic assessment and a valid spleen stiffness measurement. Among these, 13 patients were found to have OVs. Thirteen (37%) patients met the Baveno VI criteria and 14 (40%) and the Expanded Baveno VI criteria for avoiding endoscopy, while 18 (51%) patients were at low-risk based on their ElastPQ spleen stiffness (<40.2 kPa). Both Baveno VI and Expanded Baveno VI criteria showed a good sensitivity (92%, for both tests) but a scarce specificity (54% and 59%, respectively) for detecting CSPH. ElastPQ spleen stiffness showed the same sensitivity (92%), but improved specificity (77%). The performance of the Baveno VI, Expanded Baveno VI and ElastPQ spleen stiffness is summarized in **Table 15**.

The misclassification of the patients with or without oesophageal varices at endoscopy and the number of the endoscopies that could have been saved using the Baveno VI criteria, the Expanded Baveno VI criteria or ElastPQ spleen stiffness (≥40.2 kPa or <40.2 kPa) are reported in **Table 16.** The Baveno VI criteria, the Expanded Baveno VI criteria would have saved 55% and 59% of the wasted OGDs, respectively. Using ElastPQ spleen stiffness to determine the need of varices screening would increase the rate of saved endoscopies to 77%, without increasing the number of undiagnosed OVs.

The application of the quality criterion of ElastPQ spleen stiffness SD/mean ≤30% did not improved ElastPQ spleen stiffness performance (**Table 16**).

Table 15. Performance of Baveno VI criteria, Expanded Baveno VI criteria and spleen stiffness in predicting the presence of oesophageal varices in PSC.

	OVs/n	Cut-off	Se	Sp	PPV	NPV	PLR	NLR
Baveno VI	13/35	-	0.92	0.54	0.55	0.92	2.0	0.15
Expanded Baveno VI	13/35	-	0.92	0.59	0.57	0.93	2.2	0.14
ElastPQ spleen stiffness	13/35	40.2 kPa	0.92	0.77	0.71	0.94	4	0.10

Abbreviations: PSC primary sclerosing cholangitis; OVs, oesophageal varices; n, number of patients with varices; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

Table 16. Misclassification and performance of the patients with or without oesophageal varices at endoscopy using Baveno VI criteria, Expanded Baveno VI criteria and ElastPQ spleen stiffness.

	FN	FP	Spared OGDs*	Missed OVs**
Baveno VI	1	10	12/22 (55%)	1/13(8%)
Baveno VI expanded	1	9	13/22 (59%)	1/14 (7%)
ElastPQ spleen stiffness	1	5	17/22 (77%)	1/18 (6%)
ElastPQ spleen stiffness SD/mean ≤30%	1	4	14/18 (78%)	1/15 (7%)

Abbreviations: FN, false negative; FP, false positive; OVs, oesophageal varices; OGDs, oesophagogastroscopies; SD, standard deviation.

** Spared OGDs are expressed as: (number of patients who did not have OVs at endoscopy – FP) / number of patients who did not have OVs at endoscopy %.*

** Missed OGDs are expressed as: FN / number of patients who met criteria for avoiding endoscopy %.*

4.3 Non-invasive assessment of fibrosis stage in Primary Biliary Cholangitis

4.3.1 Baseline characteristics

One hundred fifty-four patients with PBC [10 males (6.5%), mean age 57 ± 11] were recruited in the study. The main characteristics of the studied population are described in **Tables 17** and **18**.

Measurements performed with both ElastPQ and F-TE were available for 152 patients, while spleen stiffness measurement was available in 97 (63%) patients.

Table 17. Clinical and biochemical characteristic of the patients affected by primary biliary cholangitis.

Number of patients	154
Age, years, mean ± SD	57 ± 11
Male sex, n (%)	10 (6.5)
AMA positivity, n (%)	130 (87.2)
Age at PBC diagnosis, years, mean ± SD	50 ± 11
PBC duration, months, median (IQR)	74 (113)
AIH overlap syndrome, n (%)	12 (7.8)
Treatment with UDCA, n (%)	112 (72.7)
UDCA responders, n (%)	55/112 (49.1)
HCC, n (%)	2 (1.3)
Ascites, n (%)	4 (2.6)

History of HE, n (%)	0 (0)
History of variceal bleeding, n (%)	1 (0.6)
OGD overall, n (%)	53 (34.4)
OGD within 12 months, n (%)	53 (34.4)
Oesophageal varices, n (%)	12 (22.6)
High risk oesophageal varices, n (%)*	4 (2.6)
Gastric varices, n (%)	0 (0)
Portal hypertensive gastropathy, n (%)	9 (5.8)
Treatment with beta-blockers, n (%)	4 (2.6)
Child-Pugh class, n (%)	
A	147 (95.5)
B	7 (4.5)
C	0 (0)
MELD score, median (IQR)	6.4 (0.7)
PBC Mayo risk score, median (IQR)	4.32 (0.76)
Biochemistry at the time of elastography**	
Bilirubin (mg/dL), median (IQR)	0.4 (0.36)
Albumin (g/dL), mean ± SD	4.3 (0.3)
AST (IU/L), median (IQR)	39 (32)
ALT (IU/L), median (IQR)	44 (52)
ALP (IU/L), median (IQR)	151 (212)
PLTs, mean ± SD	263 (88)
Serum creatinine (mg/dl), mean ± SD	0.8 ± 0.2
Serum Na, median (IQR)	141 (3)
INR, median (IQR)	1.0 (0.1)

** High-risk varices were defined as grade ≥2 oesophageal varices, or any varix with a red colour sign.*

Values are expressed as median (IQR), unless otherwise specified. Abbreviations: SD, standard deviation; AMA, anti-mitochondrial antibodies; PBC, primary biliary cholangitis; AIH, autoimmune hepatitis; UDCA, ursodeoxycholic acid; HCC, hepatocellular carcinoma; HE, hepatic encephalopathy; OGD, oesophagogastroduodenoscopy; MELD, model of end-stage liver disease; IQR, interquartile range; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; PLTs, platelets; Na, sodium; INR, International Normalized Ratio.

Table 18. Fibrosis scores, sonographic characteristics, elastography and histology in the PBC patients.

Fibrosis scores at the time of elastography	
APRI index	0.45 (0.57)
AST/ALT ratio	0.84 (0.39)
FIB-4	1.25 (0.97)
Fibrosis index score, mean ± SD	1.02 ± 1.10
King's score	7.7 (9.5)
Lok score	-4.8 (7.6)
Fibro Q score	1.9 (1.9)
GUCI score	0.43 (0.50)
Bonacini score, mean ± SD	3 ± 3
ELF score*	10.03 ± 1.04

Liver stiffness measurement by F-TE	
Probe M/XL, n (%)	149/5 (96.8/3.2)
F-TE (median, kPa)	7.7 (6.5)
F-TE IQR (kPa)	1.1 (1.4)
F-TE IQR/median%	15 (12)
ElastPQ liver stiffness	
ElastPQ liver stiffness (average, kPa)	6.6 (6.8)
ElastPQ liver stiffness SD (kPa)	1.4 (1.5)
ElastPQ liver stiffness (median, kPa)	6.68 (6.80)
ElastPQ liver stiffness SD/average (%)	20 (10)
ElastPQ liver stiffness SD/average ≤30%	132 (86.3)
ElastPQ spleen stiffness	
ElastPQ spleen stiffness failure, n (%)	57 (37)
ElastPQ spleen stiffness (average, kPa)	26.1 (13.3)
ElastPQ spleen stiffness SD (kPa)	5.5 (4.8)
ElastPQ spleen stiffness (median, kPa)	25.7 (14.6)
ElastPQ spleen stiffness SD/average (%)	21 (73)
Histology stage, n (%)**	
F0	4 (25)
F1	4 (25)
F2	4 (25)
F3	1 (6.3)
F4	3 (18.8)

Fibrosis stage assessed with F-TE as reference standard	
F0	72 (47.1)
F1	18 (11.8)
F2	16 (10.5)
F3	25 (16.3)
F4	22 (14.4)
Spleen size, cm	10.1 (2.3)
Spleen area, cm2* **	33 (17)
LSPS	0.29 (0.42)
SSPSA[§]	0.94 (1.8)
Baveno VI criteria	
Low risk, n (%)	133 (86.4)
High risk, n (%)	21 (13.6)
Expanded Baveno VI criteria	
Low risk, n (%)	140 (90.9)
High risk, n (%)	14 (9.1)

**ELF score at the time of elastography was available in 48 (31.2%) patients.*

***Liver biopsy within 12 months from elastography was available in only 16 patients.*

****Spleen area was available for 87 (56.5%) patients.*

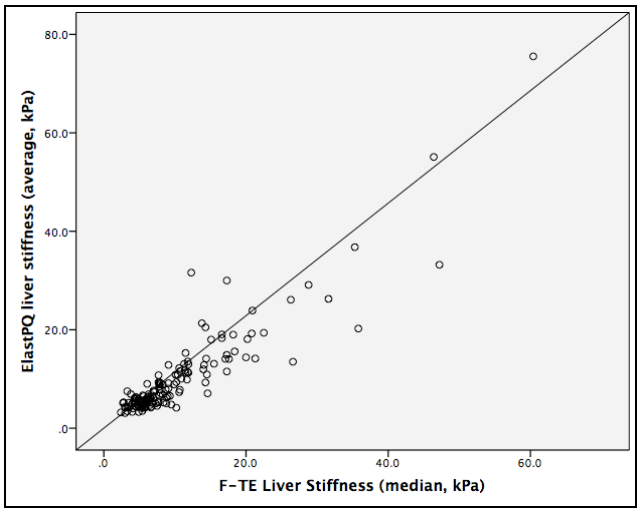
[§]*SSPSA was available for 86 (55.8%) patients.*

Values are expressed as median (IQR), unless otherwise specified. Abbreviations: APRI, AST to platelet ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase; SD, standard deviation; ELF, enhanced liver fibrosis; F-TE, transient elastography performed by FibroScan.

4.3.2 Comparison between shear wave elastography (ElastPQ) and F-TE.

ElastPQ liver stiffness demonstrated to have an excellent correlation with F-TE regardless of whether ElastPQ mean value ($p<0.0001$, Spearman's correlation coefficient 0.85; Lin's correlation coefficient 0.91, 95%CI 0.88 to 0.93) or ElastPQ median value was considered ($p<0.0001$, Spearman's correlation coefficient 0.83; Lin's correlation coefficient 0.87, 95%CI 0.83 to 0.91) (**Figure 32 A** and **B**).

A



B

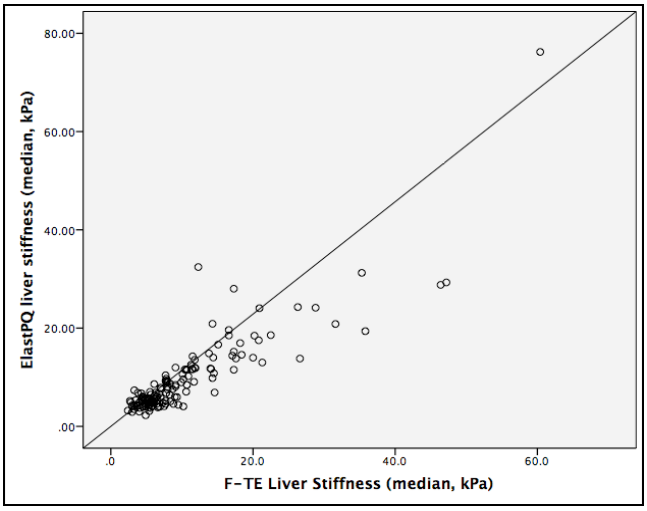


Figure 32. *Linear correlation between F-TE and liver ElastPQ mean (A) or median (B) in the overall PBC population.*

The comparison of the correlation's coefficients for mean ElastPQ and median ElastPQ liver stiffness was not statistically significant (p=0.140). The application of quality criterion (ElastPQ SD/mean ≤30%), which was met by 132 patients (86%) did not influence the test accuracy (p<0.0001, Spearman's correlation coefficient 0.83; Lin's correlation coefficient 0.90, 95%CI 0.87 to 0.91) (**Figure 33**).

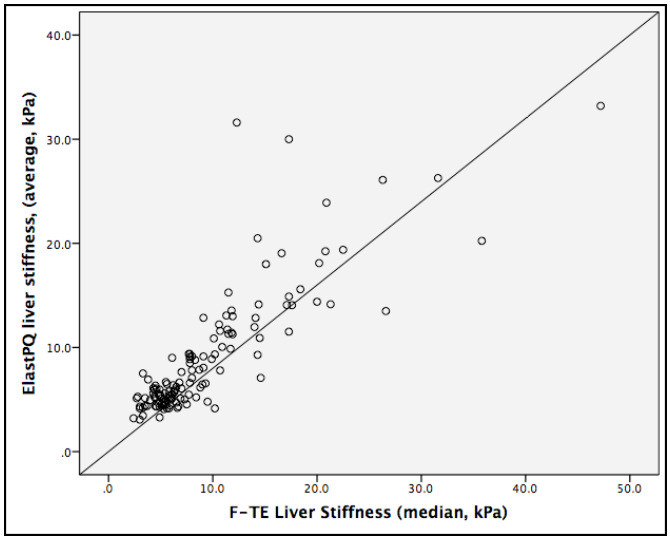
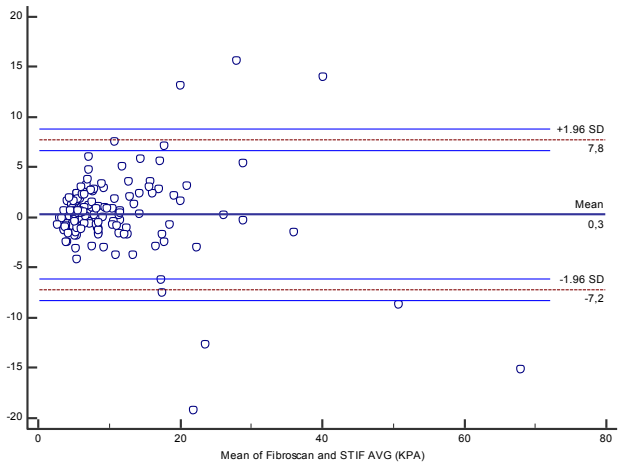


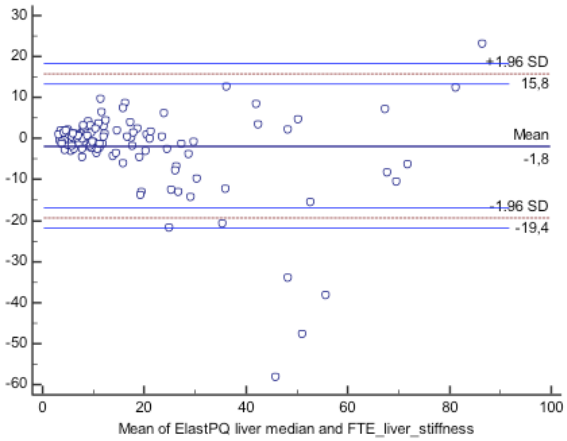
Figure 33. *Linear correlation between F-TE and ElastPQ liver stiffness in the subgroup with ElastPQ SD≤30%.*

Bland-Altman plot agreement analyses are shown in the figures below (**Figure 34 A, B** and **C**).



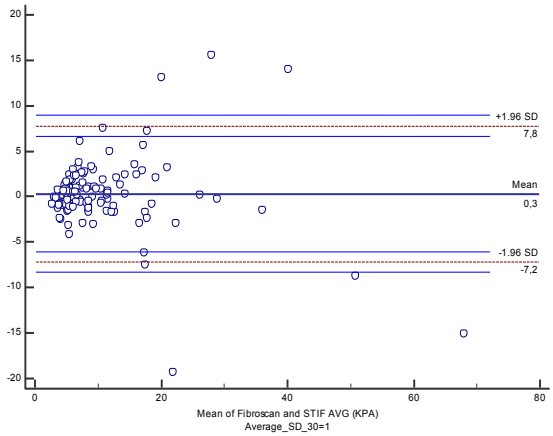
Sample size	152
Arithmetic mean	0,2730
95% CI	-0,3394 to 0,8855
P (H_0: Mean=0)	0,3798
Standard deviation	3,8216
Lower limit	-7,2173
95% CI	-8,2663 to -6,1684
Upper limit	7,7634
95% CI	6,7144 to 8,8124

Figure 34 A. *Bland-Altman plot agreement analysis between F-TE and mean ElastPQ in the overall PBC population.*



Sample size	152
Arithmetic mean	-1,7920
95% CI	-3,2313 to -0,3528
P (H_0: Mean=0)	0,0150
Standard deviation	8,9806
Lower limit	-19,3940
95% CI	-21,8590 to -16,9290
Upper limit	15,8099
95% CI	13,3449 to 18,2750

Figure 34 B. *Bland-Altman plot agreement analysis between F-TE and median ElastPQ in the overall PBC population.*



Sample size	130
Arithmetic mean	0,3010
95% CI	-0,3616 to 0,9636
P (H_0: Mean=0)	0,3705
Standard deviation	3,8186
Lower limit	-7,1834
95% CI	-8,3187 to -6,0481
Upper limit	7,7854
95% CI	6,6501 to 8,9207

Figure 34 C. *Bland-Altman plot agreement analysis between F-TE and mean ElastPQ in the subgroup with ElastPQ SD/mean≤30%.*

The continuous line is the mean of the differences. The dashed grey lines define the 95% limits of agreement, with the blue lines representing the confidence interval (CI) (mean of the difference (±2SD)).

4.3.3. Correlation between liver elastography and surrogate markers of liver fibrosis.

Liver ElastPQ, as well as F-TE, showed a significant but moderate correlation with APRI index, FIB-4, King's score, GUCI and ELF score (P<0.001). A poor but still significant correlation was found with Fibro Q, Fibrosis index, Lok score and Bonacini score (P<0.001), while (as for PSC) no correlation was detected with AST/ALT ratio (**Table 18**). The best Spearman's coefficient (rho) was found for the ELF score (0.60 with regards to F-TE, 0.58 with regards to ElastPQ) and the APRI index (0.55 with regards to F-TE, 0.57 with regards to ElastPQ). The correlation with the ELF score was limited by the small number of patients with an available ELF test at the time of elastography.

Table 18. Correlation between liver elastography and surrogate markers of liver fibrosis.

		APRI	AST/ALT	FIB4	King's	Fibrosis index	Lok score	Fibro Q	GUCI	Bonacini	ELF
F-TE	*r*	0.55	-0.05	0.43	0.53	0.35	0.30	0.23	0.54	0.30	0.60
	P	<0.001	0.521	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	<0.001
ElastPQ liver (mean)	*r*	0.57	-0.04	0.40	0.55	0.32	0.24	0.17	0.56	0.24	0.58
	P	<0.001	0.592	<0.001	<0.001	<0.001	0.002	0.036	<0.001	0.002	<0.001
ElastPQ liver (median)	*r*	0.57	-0.06	0.39	0.54	0.31	0.23	0.15	0.56	0.23	0.60
	P	<0.001	0.445	<0.001	<0.001	<0.001	0.006	0.068	<0.001	0.004	<0.001

Abbreviations: APRI, AST to Platelet Ratio Index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GUCI, Göteborg University Cirrhosis Index; ELF, enhanced liver fibrosis (score).

4.3.4 Correlation between elastography, prognostic scores and serum markers of inflammation and cholestasis.

Liver elastography (measured both with FibroScan and ElastPQ) and ElastPQ spleen elastography were demonstrated to have a fair statistically significant correlation with the MELD score (P<0.001 for all the correlations; Spearman's rho coefficient 0.28, 0.30 and 0.36 for F-TE, liver ElastPQ and spleen ElastPQ, respectively) and a poorer but still significant correlation with the PBC Mayo risk score (rho = 0.23, P=0.004 for F-TE; rho = 0.24, P=0.002 for liver ElastPQ and rho = 0.28, P=0.006 for spleen ElastPQ) (**Table 19**). All the elastographic parameters showed a moderate significant correlation with ALT, AST and bilirubin. F-TE and liver ElastPQ were also significantly correlated with the levels of ALP, again with modest correlation coefficients (rho=0.30 for F-TE, rho=0.38 for ElastPQ, P<0.001 for both the correlations). On the contrary, ALP levels were not correlated with the spleen stiffness (**Table 19**).

Table 19. Correlation between liver elastography, prognostic scores and serum markers of inflammation and cholestasis.

		MELD	PBC Mayo RS	ALT	AST	Bilirubin	ALP
F-TE	*r*	0.28	0.23	0.39	0.51	0.44	0.30
	P	<0.001	0.004	<0.001	<0.001	<0.001	<0.001
ElastPQ liver (mean)	*r*	0.30	0.24	0.45	0.54	0.46	0.38
	P	0.001	0.002	<0.001	<0.001	<0.001	<0.001
ElastPQ liver (median)	*r*	0.28	0.24	0.47	0.41	0.50	0.41
	P	<0.001	0.003	<0.001	<0.001	<0.001	<0.001
ElastPQ spleen (mean)	*r*	0.36	0.28	0.14	0.36	0.44	0.19
	P	<0.001	0.006	0.18	<0.001	<0.001	0.06
ElastPQ spleen (median)	*r*	0.37	0.31	0.15	0.39	0.43	0.18
	P	<0.001	0.002	0.15	<0.001	<0.001	0.78

Abbreviations: MELD, model of end-stage liver disease; PBC, primary biliary cholangitis; RS, risk score; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase, r, rho.

4.3.5 Performance of liver ElastPQ in differentiating liver fibrosis stages.

Since in most cases biochemical and serological profile are sufficient to achieve a diagnosis of PBC [108], a liver biopsy in these patients is performed only rarely in routine clinical practice. For this reason, histology within 12 months from elastography was available only for 16 patients, in this cohort.

As in PSC, also in PBC the ability of FibroScan to differentiate fibrosis stages has been validated against liver histology (gold standard). Due to the lack of available

biopsies, for the purpose of this study F-TE has been used as a surrogate of histological fibrosis, using the previously validated thresholds of liver stiffness as the reference standard [13].

The median value of liver ElastPQ was significantly different according to the fibrosis stage as defined by F-TE categories (P=0.001) (**Figure 35**).

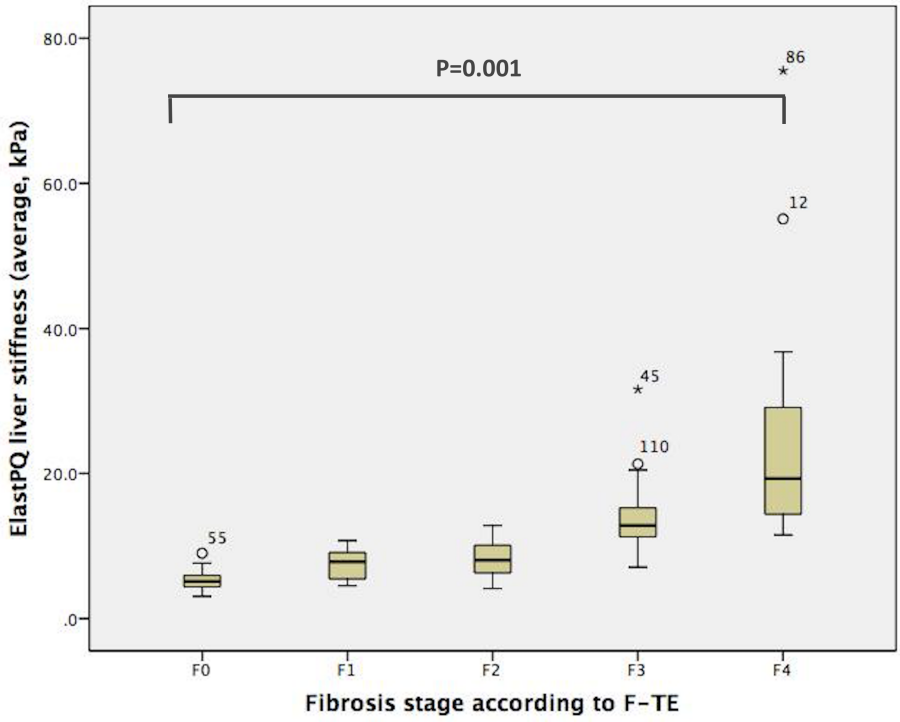


Figure 35. *Distribution of ElastPQ values according to fibrosis stage as defined by FibroScan.*

In the pairwise comparison, after Bonferroni adjustment, a statistically significant difference did not exist only when comparing two consecutive stages (with the exception of F0 vs. F1), but was present in all other inter-stage comparisons.

The Kruskall-Wallis test revealed a statistical significance also when evaluating the differences of median values of liver elastography (measured both by F-TE and ElastPQ) across the stages determined by histology. However, due to the very small

number of available biopsies, these results are reported only with a descriptive purpose (**Figure 36 A** and **B**).

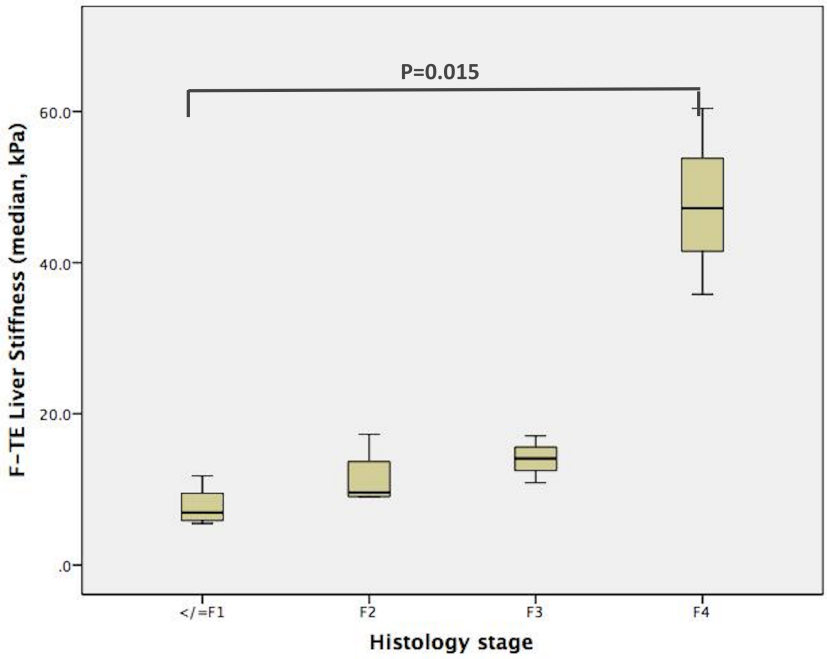


Figure 36 A. *Distribution of F-TE values according to histological stage.*

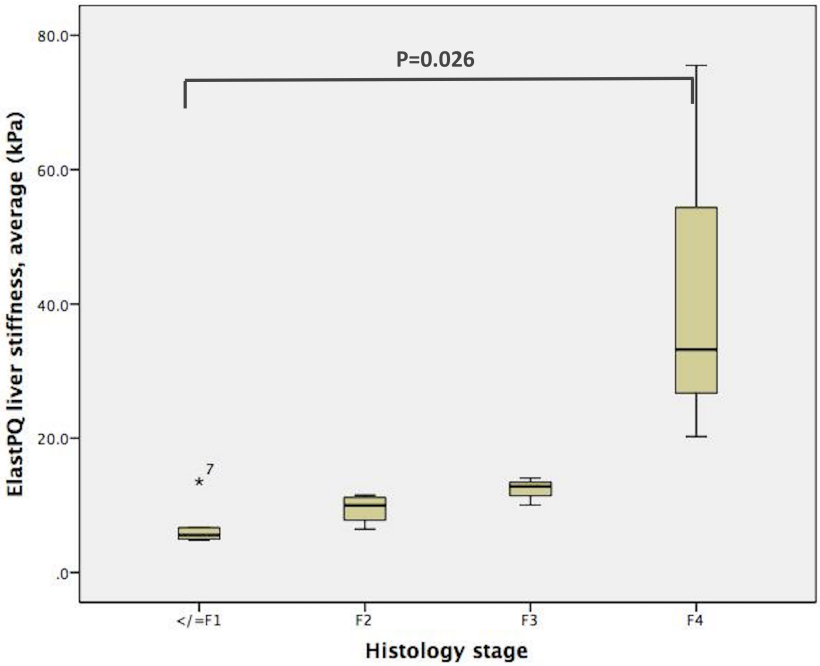


Figure 36 B. *Distribution of ElastPQ liver stiffness values according to histological stage.*

AUROC curves and optimal liver ElastPQ cut-offs were obtained for each stage of fibrosis (**Figures 37-40**). AUROCs (95%CI) for liver stiffness measured by ElastPQ were 0.93 (0.89–0.97), 0.94 (0.90–0.98), 0.98 (0.97–0.99), 0.97 (0.94–0.99) for fibrosis stages F≥1, F≥2, F≥3 and F=4, respectively. Optimal cut-off values were 7.7 kPa (80% sensitivity, 99% specificity), 8.9 kPa (84% sensitivity, 93% specificity), 9.6 kPa (94% sensitivity, 95% specificity) and 13.3 kPa (96% sensitivity, 93% specificity) for mild, moderate, severe fibrosis and cirrhosis, respectively (**Table 20**).

Similar results were obtained when the patients with AIH overlap where excluded from the analysis. The application of the quality criterion ElastPQ SD/mean ≤30% did not improved significantly the performance of ElastPQ liver stiffness in discriminating fibrosis stages (**Table 21**).

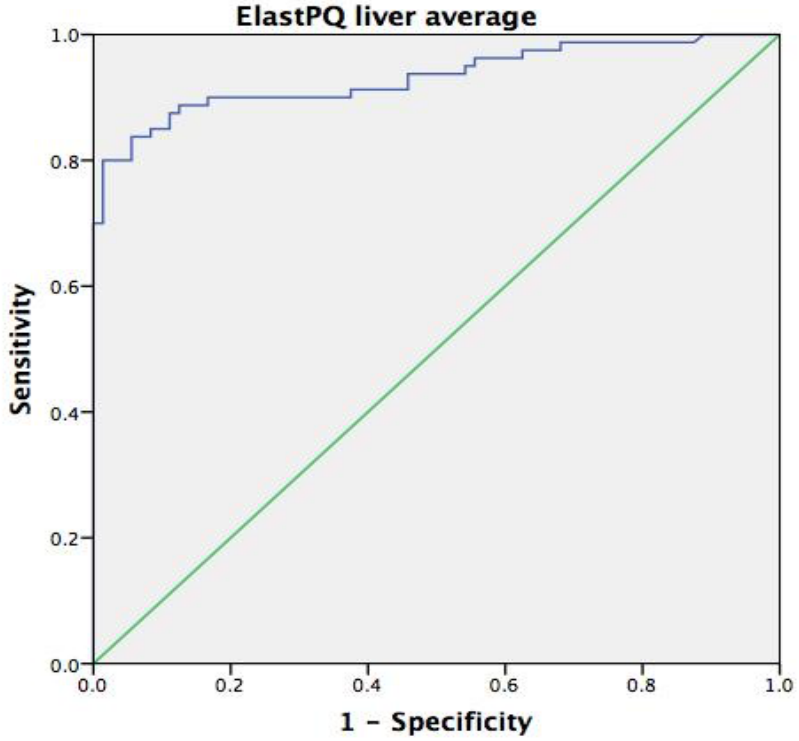


Figure 37. *Receiver operating characteristic analysis of the ability of liver ElastPQ to predict mild fibrosis (F≥1) in PBC.*

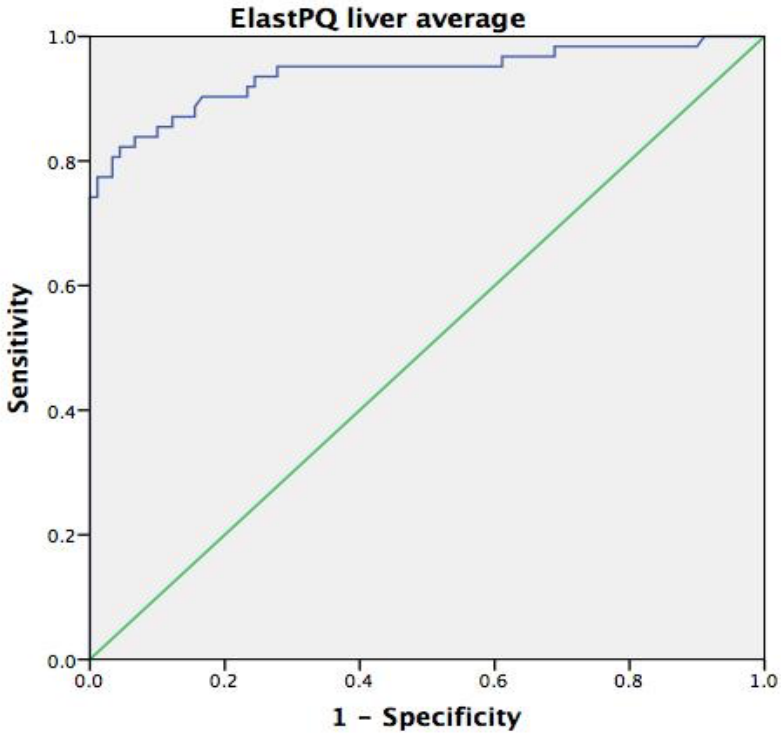


Figure 38. *Receiver operating characteristic analysis of the ability of liver ElastPQ to predict moderate fibrosis (F≥2) in PBC.*

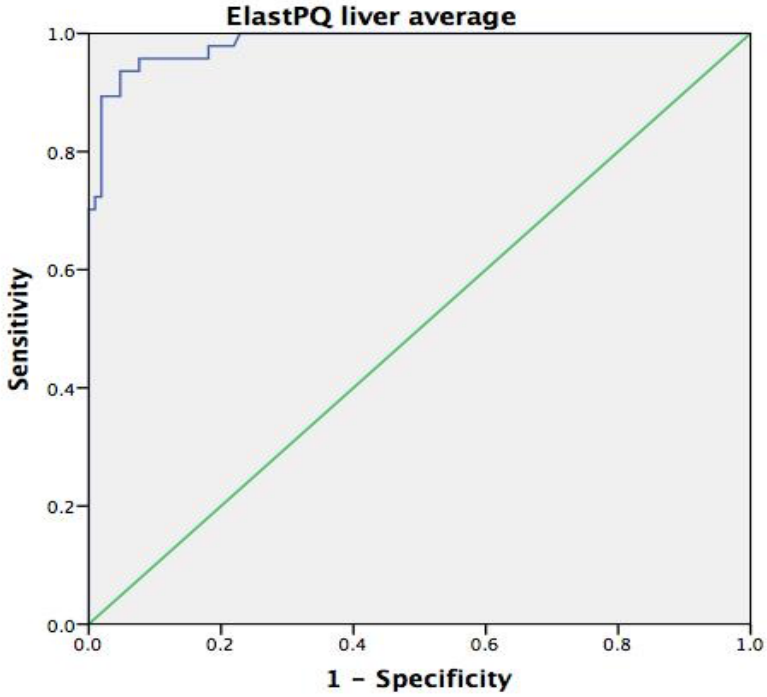


Figure 39. *Receiver operating characteristic analysis of the ability of liver ElastPQ to predict severe fibrosis (F≥3) in PBC.*

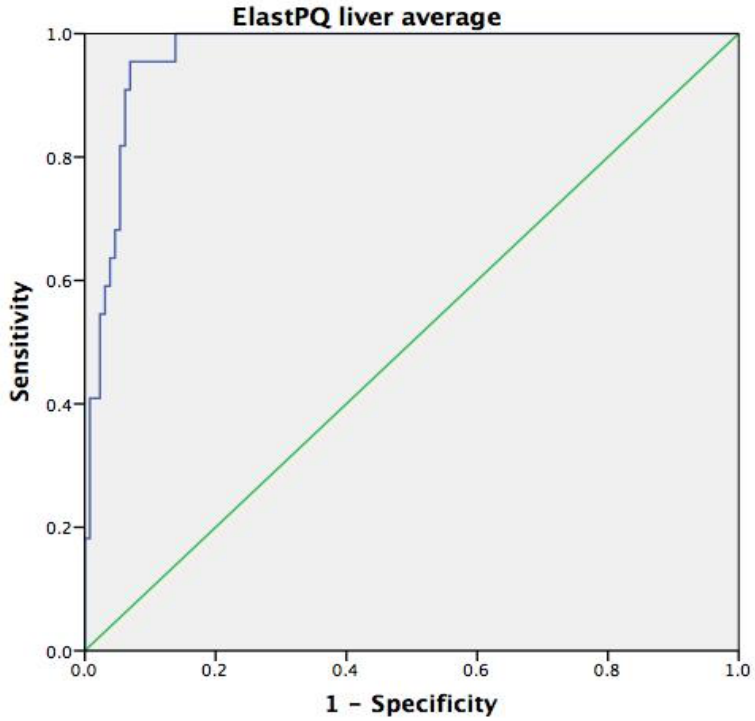


Figure 40. *Receiver operating characteristic analysis of the ability of liver ElastPQ to predict cirrhosis (F=4) in PBC.*

Table 20. Performance of ElastPQ liver stiffness in differentiating liver fibrosis stages (according to F-TE) in PBC.

Stage	N.	Cut-off (kPa)	Se	Sp	PPV	NPV	PLR	NLR	AUROC (95%CI)
≥ F1	80	7.7	0.80	0.99	0.99	0.81	80	0.20	0.93 (0.89-0.97)
≥ F2	62	8.9	0.84	0.93	0.90	0.89	12	0.17	0.94 (0.90-0.98)
≥ F3	47	9.6	0.94	0.95	0.90	0.97	18.8	0.06	0.98 (0.97-0.99)
F4	22	13.3	0.96	0.93	0.70	0.99	13.7	0.04	0.97 (0.94-0.99)

Abbreviations: kPa, kilopascal; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; AUROC, area under receiver operating characteristic curve; CI, confidence interval.

Table 21. Performance of ElastPQ liver stiffness in differentiating liver fibrosis stages (according to F-TE) in PBC, when the quality criterion ElastPQ SD/mean ≤30% was applied.

Stage	N.	Cut-off (kPa)	Se	Sp	PPV	NPV	PLR	NLR	AUROC (95%CI)	P*
≥ F1	66	7.7	0.82	0.99	0.98	0.84	82	0.18	0.94 (0.89-0.98)	*ns*
≥ F2	51	8.9	0.84	0.94	0.89	0.90	14	0.17	0.94 (0.90-0.99)	*ns*
≥ F3	39	9.6	0.92	0.97	0.92	0.97	30.7	0.08	0.98 (0.96-1.0)	*ns*
F4	17	13.3	0.94	0.94	0.70	0.99	15.7	0.06	0.97 (0.94-0.99)	*ns*

Abbreviations: kPa, kilopascal; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; AUROC, area under receiver operating characteristic curve; CI, confidence interval.

**P value refers to the comparison between the AUROC obtained for the overall population and the AUROC obtained when the quality criterion ElastPQ SD/mean ≤30% was applied.*

Table 22 reports the misclassification in staging liver fibrosis (defined by F-TE cut-offs), using the cut-offs obtained for ElastPQ liver stiffness.

Table 22. Misclassification in staging liver fibrosis using ElastPQ liver stiffness.

Fibrosis stage (F-TE)	FN	FP	TOT
ElastPQ mean (Overall)			
F≥1	16/80 (20%)	1/71 (1%)	21%
F≥2	10/61 (16%)	6/89 (7%)	23%
F≥3	3/47 (6%)	5/105 (5%)	11%
F=4	1/22 (4%)	9/130 (7%)	11%
Total misclassified	30/152 (20%)	32/152 (14%)	24%
ElastPQ mean (SD/mean ≤30%)			
F≥1	12/66 (18%)	1/64 (2%)	20%
F≥2	8/50 (16%)	5/79 (6%)	22%
F≥3	3/39 (8%)	3/92 (3%)	11%
F=4	1/17 (6%)	7/114 (6%)	12%
Total misclassified	24/131 (18%)	16/131 (12%)	30%

Abbreviations: F-TE, liver transient elastography performed with FibroScan; FN, false negative; FP, false positive; TOT, total; SD, standard deviation.

4.3.6 Factors influencing the discrepancy between F-TE and ElastPQ.

The confounding factors potentially influencing the discrepancy between F-TE and ElastPQ values were investigated.

Statistically significant variables associated with a difference between the two techniques of ± 2 kPa, at the univariate analysis, were the Mayo risk score, bilirubin, platelet count, and the diagnosis of cirrhosis as per F-TE values (**Table 23**). On multivariate analysis, only the diagnosis of cirrhosis maintained its statistical significance (OR 9.017, 95%CI 2.90-27.98, P<0.001).

The results were not significantly different when the considered delta liver stiffness between F-TE and ElastPQ measurement was 5 kPa or 10 kPa (data not shown).

Table 23. Factors associated with a difference of ± 2 kPa between liver stiffness measured by F-TE and ElastPQ in the PBC cohort.

	Univariate			Multivariate		
	F-TE-ElastPQ liver < 2 kPa	**F-TE ElastPQ liver ≥2 kPa**	**P value**	**OR**	**95% CI**	**P**
PBC Mayo RS	4.23	4.46	0.028			
AIH overlap	8/103 (8%)	4/49(8%)	0.581			
Gender (male)	7/103 (7%)	3/49 (6%)	0.590			
Age, years ± SD	57 ± 11	57 ± 11	0.987			
BMI ≥30 kg m^2	13/103 (13)	6/49 (12)	1.000			
Bilirubin (mg/dL)	0.4 (0.3)	0.5 (0.4)	0.006			
ALT (IU/L)	42 (54)	49 (45)	0.191			
AST (IU/L)	34 (29)	44 (35)	0.104			
ALP (IU/L)	151 (218)	151 (208)	0.573			
Platelets/mmc	270 (100)	246 (110)	0.029			
Cirrhosis (F-TE = F4)	5/103 (5%)	17/49 (35%)	<0.001	*9.017*	*2.90-27.98*	<0.001

Numerical variables are expressed as median (IQR), dichotomous variables are expressed as n (%).

Abbreviations: F-TE, liver elastography performed with FibroScan; kPa, kilopascal; OR, odd ratio; CI, confidence interval; PBC, primary biliary cholangitis; RS, risk score; AIH, autoimmune hepatitis; SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

4.4 Non-invasive assessment of portal hypertension in PBC.

Predictors of CSPH were investigated in the subgroup of patients who had an upper-GI endoscopy within 12 months from the date of the elastographic assessment. This group consisted of 53 patients. OVs were detected in 12 cases, and among these, only four presented varices at high-risk of bleeding.

The comparison between the groups of patients with and without OVs with regards to clinical, biochemical and elastographic characteristics are reported in **Table 24**. In particular, median values of F-TE, ElastPQ liver stiffness, ElastPQ spleen stiffness and LSPS were significantly higher in patients with OVs compared to those without OVs (**Figures 41-44**). Logistic regression analysis was performed in order to identify factors predicting the presence of OVs. At univariate analysis, statistically significant variables associated with the presence of OVs were: diagnosis of cirrhosis, Child-Pugh score, MELD score, PBC Mayo risk score, spleen area and spleen longitudinal diameter, platelet count, albumin, AST, ALT, INR, F-TE, liver and spleen ElastPQ, LSPS and SSPSA (**Table 25**). Different models of multivariate logistic regression analysis were performed including the variables statistically significant in the univariate analysis. Due to a high degree of collinearity (Spearman's rho=0.80, P<0.001), ElastPQ spleen stiffness and LSPS were not included in the same model. ElastPQ spleen stiffness remained the only independent predictor of CSPH in all the models (OR 1.101; CI 1.14-1.20; p <0.022) (**Table 25**), regardless of whether the Mayo risk score, MELD or Child-Pugh score was included as indicator of liver disease severity, or if F-TE or ElastPQ were included as measure of liver stiffness. ElastPQ spleen stiffness remained the only variable independently associated with OVs also when the multivariate analysis was adjusted for the presence of cirrhosis.

Table 24. Comparison between patients with and patients without varices.

	No OVs	OVs	P
Age	58±12	54±14	0.265
Cirrhosis	11 (26.8%)	8 (72.7%)	0.011
PBC Mayo RS	4.5±0.6	4.1±1.1	0.099
Child-Pugh score	5 (0)	5 (2)	0.003
MELD	6.4 (1.1)	8.2 (3.4)	0.003
Spleen area, cm^2	31 (32)	67 (59)	0.017
Spleen LD, cm	10.3 (3.8)	13.4 (5.0)	0.001
Bilirubin mg/dL	0.4 (0.3)	1.2(1.0)	0.005
AST (IU/L)	40 (45)	83 (96)	0.004
ALP (IU/L)	156 (202)	237 (303)	0.758
Platelets/mmc	263±92	145±65	<0.001
Albumin g/dL	4.3±0.3	4.0±0.5	0.067
INR	1.0 (0.1)	1.1 (0.2)	<0.001
F-TE liver stiffness, kPa	10.9 (11.3)	28.8 (31.8)	0.001
ElastPQ liver stiffness, kPa	11.3 (9.6)	27.7 (22.3)	0.001
ElastPQ spleen stiffness, kPa	31.6 (14.1)	51.8 (26.5)	<0.000
LSPS	28 (10.8)	90.7 (46.2)	<0.001
SSPSA	0.44 (0.59)	2.9 (2.8)	0.001
ELF score*	1.6 (5.6)	12.1 (12.1)	0.004

Values are expressed as median (IQR) or mean ± SD, according to distribution.

**ELF available only in 18 patients with OGD within 1 year (and only in 3 with OVs).*

Abbreviations: OVs, oesophageal varices; PBC, primary biliary cholangitis; RS, risk score; MELD, model of end-stage liver disease; LD, longitudinal diameter; AST, aspartate aminotransferase; ALP, alkaline phosphatase; INR, international normalized ratio; F-TE, liver elastography performed with FibroScan; kPa, kilopascal; ELF, enhanced liver fibrosis score.

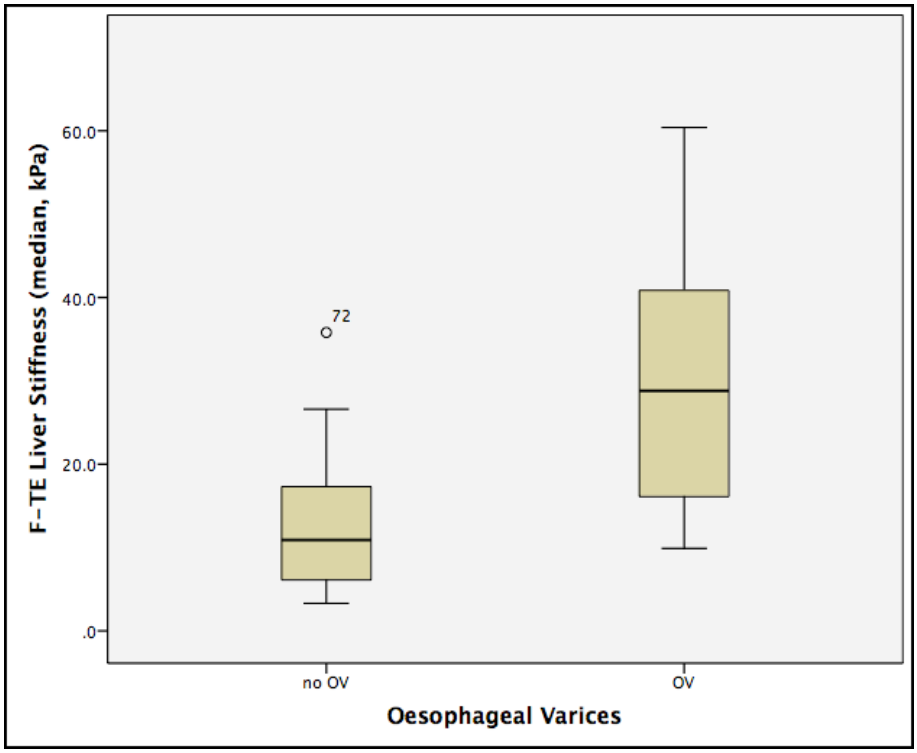


Figure 41. *Difference in the median values of F-TE between patients with and without oesophageal varices (P=0.000).*

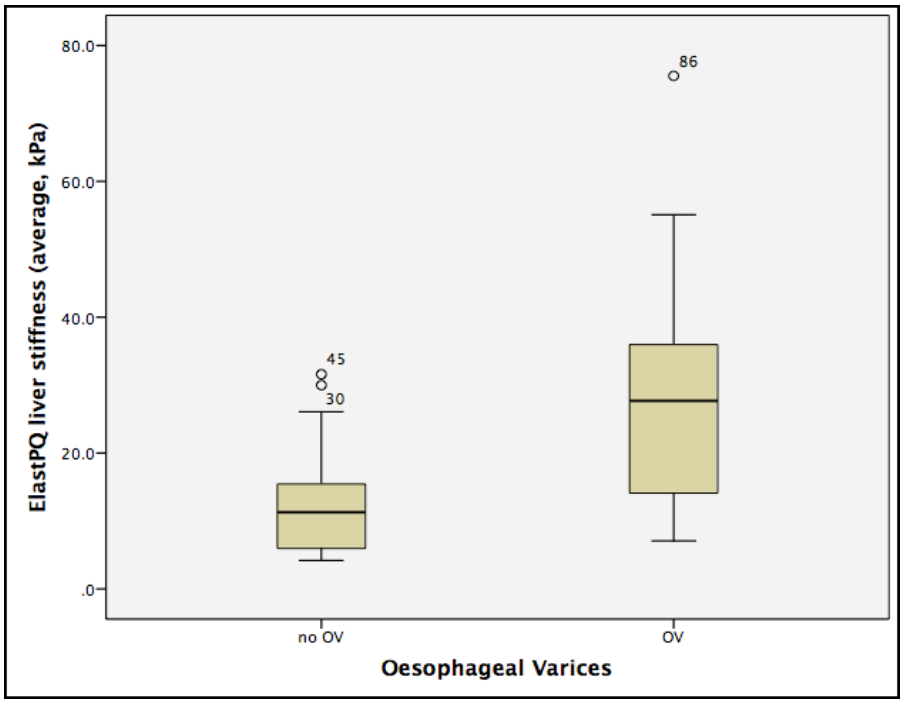


Figure 42. *Difference in the median values of ElastPQ liver stiffness between patients with and without oesophageal varices (P=0.000).*

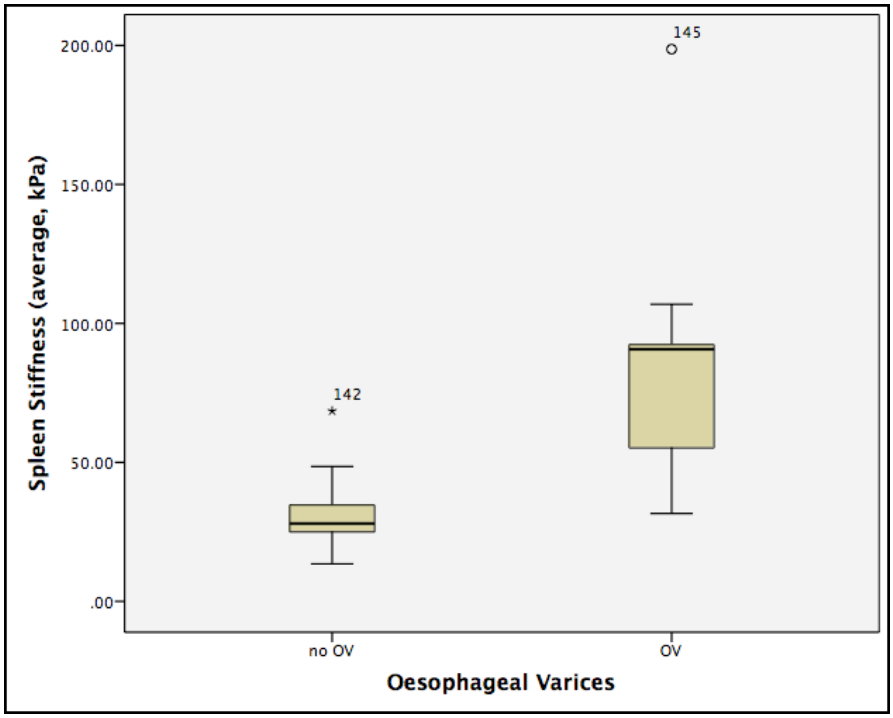


Figure 43. *Difference in the median values of ElastPQ spleen stiffness between patients with and without oesophageal varices (P<0.000).*

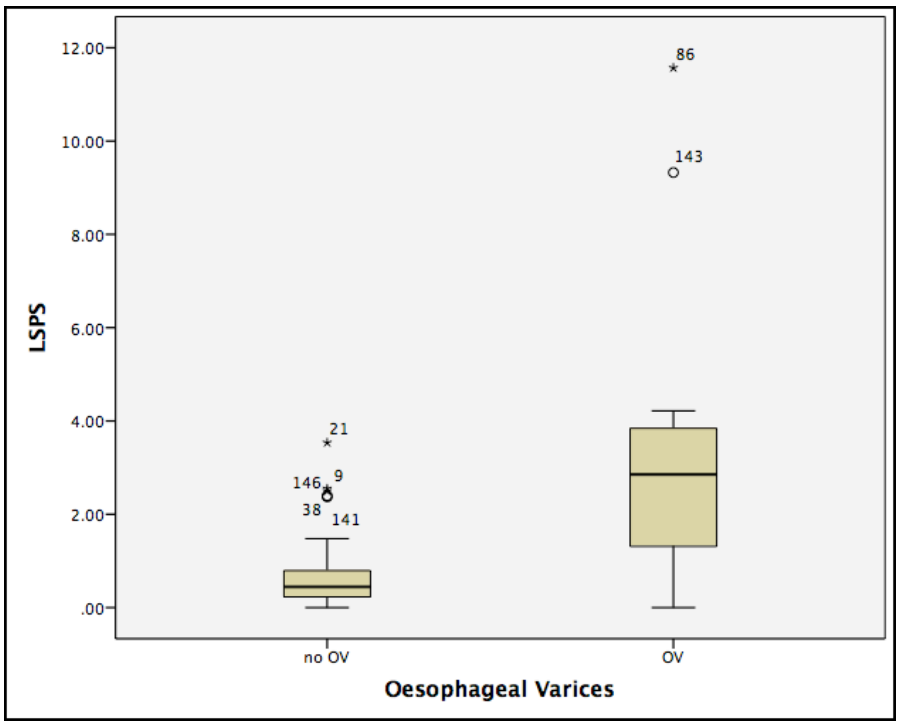


Figure 44. *Difference in the values median of LSPS score between patients with and without oesophageal varices (P<0.000).*

Table 25. Univariate and multivariate logistic regression analysis of factors potentially associated with the presence of clinically significant portal hypertension in PBC patients with an OGD within 12 months.

	Univariate			Multivariate		
	OR	**95% CI**	**P**	**OR**	**95% CI**	**P**
PBC Mayo RS	2.595	1.103-6.104	0.029			
Child-Pugh score	3.135	1.268-7.750	0.013			
MELD	1.569	1.057-2.329	0.025			
Spleen area, cm2	1.040	1.007-1.075	0.017			
Spleen LD, cm	1.442	1.128-1.854	0.004			
Bilirubin mg/dL	1.751	1.889-3.448	0.105			
AST (IU/L)	1.029	0.010-1.050	0.003			
ALP (IU/L)	1.000	0.997-1.004	0.863			
Platelets/mmc	0.980	0.968-0.992	0.001			
Albumin g/dL	0.105	0.015-0.752	0.025			
INR	167244E	1010-2768…	0.002			
F-TE liver stiffness, kPa	1.142	1.049-1.242	0.002			
ElastPQ liver stiffness, kPa	1.133	1.044-1.230	0.003			
ElastPQ spleen stiffness, kPa	1.124	1.035-1.220	0.005	*1.135*	*1.010-1.276*	*0.033*
LSPS	3.593	1.650-7.822	0.001			
SSPSA	1.217	1.026-1.443	0.024			
ELF score*	1.136	0.327-3.942	0.841			

**ELF available only in 18 patients with OGD within 1 year (and only in 3 with OVs).*

Abbreviations: PBC, primary biliary cholangitis; OGD, oesophagogastroduodenoscopy; OR, odd ratio; CI, confidence interval; RS, risk score; MELD, model of end-stage liver disease; LD, longitudinal diameter; AST, aspartate aminotransferase; ALP, alkaline phosphatase; INR, international normalized ratio; F-TE, liver elastography performed with FibroScan; kPa, kilopascal; ELF, enhanced liver fibrosis score.

The ability of F-TE, liver ElastPQ and spleen stiffness measured with ElastPQ in predicting the presence of oesophageal varices was assessed. AUROC curves were obtained for each elastographic technique, as well as for the LSPS and all the available non-invasive scores of liver fibrosis (**Tables 26** and **27**). The best cut-off value for predicting the presence of OVs was then calculated for each curve, and the diagnostic accuracy was analysed. The performance of ELF score was not evaluated in this cohort owing to the very small number of patients with available ELF test and the subsequent unreliability of the results.

Table 26. Performance of the non-invasive scores of liver fibrosis in predicting the presence of oesophageal varices in PBC.

Test	OVs/n	AUROC (95% CI)
APRI	12/53	0.890 (0.787-0.993)
AST/ALT	12/53	0.552 (0.363-0.740)
FIB-4	12/53	0.851 (0.707-0.994)
King's score	12/53	0.892 (0.766-1.000)
Fibrosis Index	12/53	0.860 (0.743-0.977)
Lok score	12/53	0.880 (0.771-0.989)
Fibro Q	12/53	0.772 (0.619-0.926)
GUCI	12/53	0.915 (0.819-1.000)
Bonacini score	12/53	0.850 (0.735-0.964)

Abbreviations: APRI, aspartate aminotransferase-to- platelet ratio index, AST to Platelet Ratio Index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GUCI, Göteborg University Cirrhosis Index.

Table 27. Performance of liver stiffness (F-TE and ElastPQ), spleen stiffness and LSPS in predicting the presence of oesophageal varices in PBC.

Test	OVs/n	Se	Sp	PPV	NPV	PLR	NLR	Cut-off value (kPa)	AUROC (95% CI)
F-TE liver	11/52	0.91	0.66	0.42	0.96	2.7	0.14	14.3	0.84 (0.71-0.97)
ElastPQ liver	12/52	0.83	0.65	0.42	0.93	2.4	0.26	13.8	0.81 (0.66-0.96)
ElastPQ spleen	9/40	0.89	0.97	0.89	0.97	29.7	0.11	50.1	0.95 (0.88-1.0)
LSPS	11/50	0.91	0.85	0.63	0.97	6.06	0.11	1.22	0.90 (0.79-1.0)

Abbreviations: PBC primary biliary cholangitis; F-TE, transient elastography performed with FibroScan; LSPS, liver stiffness X spleen diameter/platelet count; OVs, oesophageal varices; n, number of patients; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; AUROC, area under receiver operating characteristic curve; CI, confidence interval.

Both F-TE and ElastPQ liver stiffness showed good ROC curves, having AUROCs (95%CI) of 0.84 (0.71-0.97) and 0.81 (0.66-0.96), respectively. The best cut-off was 14.3 kPa for F-TE and 13.8 kPa for ElastPQ liver stiffness. However, due to quite low values of specificity, although both techniques had a good NPV, their PPV was scarce (<50%). ElastPQ spleen stiffness, instead, revealed an excellent ability in detecting CSPH, preforming better than all the other tests. The AUROC for ElastPQ spleen stiffness was 0.95 (95%CI 0.88-1.0), sensitivity 89%, specificity 97%, with an optimal cut-off of 50.1 kPa (PPV 0.89, NPV 0.97). Also LSPS was found to have a very good capacity to detect the presence of OVs (AUROC 0.90, 95%CI 0.79-1.0, Se 91%, Sp 85%), although its PPV was modest (0.63) (**Table 27, Figure 45**).

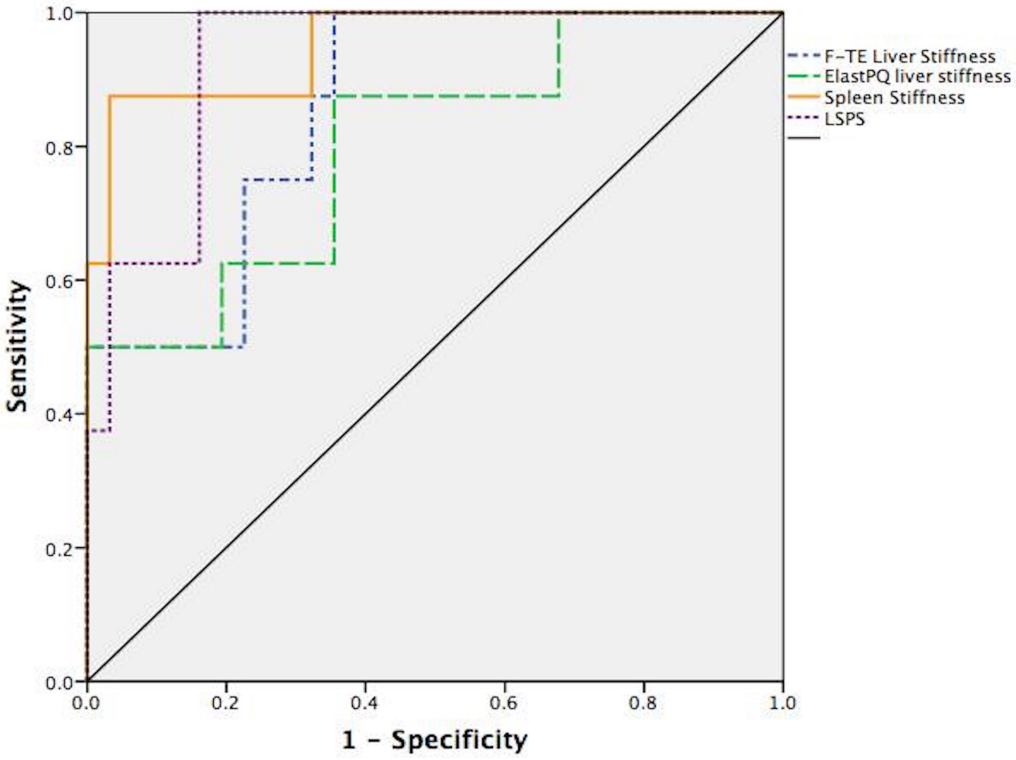


Figure 45. *Receiver operating characteristic analysis of the ability of F-TE, ElastPQ liver stiffness, ElastPQ spleen stiffness and LSPS to predict the presence of oesophageal varices in PBC.*

The AUROC for ElastPQ spleen stiffness was significantly different from the areas obtained for F-TE and ElastPQ liver stiffness (P=0.001 and P=0.002, respectively), while no statistically significant difference was identified between the areas for ElastPQ spleen stiffness and LSPS (P=0.753) (**Table 28**).

The AUROC for ElastPQ liver stiffness did not improve significantly when the quality criterion SD/mean ≤30% was applied (data not shown).

Table 28. Pairwise comparisons of ROC curves for F-TE, liver ElastPQ, spleen ElastPQ and LSPS.

Variables	Difference between areas	P value
ElastPQ spleen stiffness *vs.* F-TE	0.392	0.001
ElastPQ spleen stiffness *vs.* ElastPQ liver stiffness	0.425	0.002
ElastPQ spleen stiffness *vs.* LSPS	0.017	0.753
F-TE *vs.* ElastPQ liver stiffness	0.033	0.446
F-TE *vs.* LSPS	0.375	<0.000
ElastPQ liver stiffness *vs.* LSPS	0.408	<0.000

Abbreviations: F-TE, transient elastography performed with FibroScan; LSPS, liver stiffness X spleen diameter/platelet count.

Among the other non-invasive diagnostic tests, GUCI (AUROC 0.91, 95%CI 0.82-1.00), APRI (AUROC 0.89, 95%CI 0.79-0.99), King's score (AUROC 0.89, 95%CI 0.77-1.00) and Lok score (AUROC 0.88, 95%CI 0.77-0.99) showed a good accuracy in predicting the presence of OVs when evaluated in the overall cohort of 53 patients with OGD within 12 months (**Table 26**).

When the ROC analysis was performed in the sub-population of the patients who had spleen stiffness and spleen diameter measurement (n. 38, 8 with OVs), ElastPQ spleen stiffness showed to be the test with the best performance in predicting OVs, with an AUROC of 0.95 (95%CI 0.86-1.00), followed by GUCI (AUROC 0.94, 95%CI 0.87-1.00), King's score (AUROC 0.94, 95%CI 0.85-1.00) and LSPS (AUROC 0.93, 95%CI 0.85-1.00) (**Figure 46**).

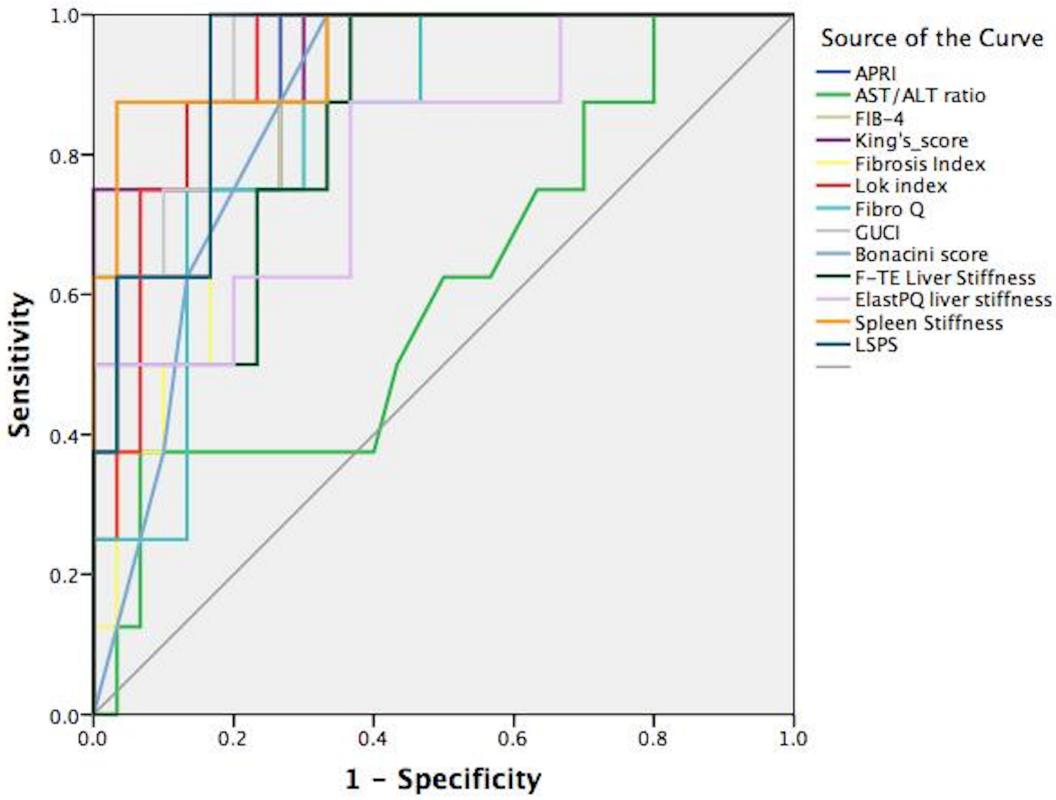


***Figure 46**. Receiver operating characteristic analysis of the ability of the non-invasive tests of liver fibrosis, liver and spleen elastography to predict the presence of oesophageal varices.*

Abbreviations: APRI, aspartate aminotransferase-to-platelet ratio index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GUCI, Göteborg University Cirrhosis Index; LSPS, liver stiffness X spleen diameter/platelet count.

ElastPQ spleen stiffness performed better than F-TE, ElastPQ liver stiffness and LSPS also when the ability of the non-invasive tests to detect patients with HRVs was examined, with a best cut-off of 53.4 kPa (AUROC = 0.94, 95%CI 0.87-1.0, Se 100%, Sp 89%) (**Figure 47**). However, the number of HRVs in this group was very low (n.4), therefore these results should be interpreted with caution.

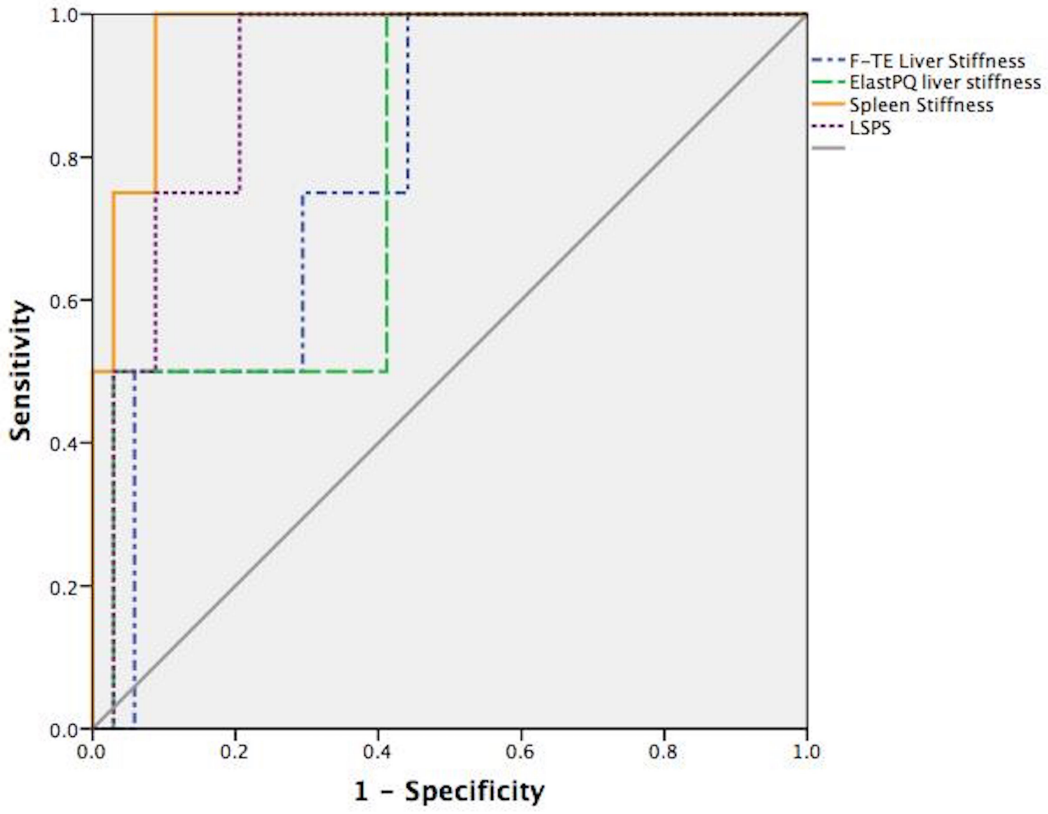


Figure 47. *Receiver operating characteristic analysis of the ability of F-TE, ElastPQ liver stiffness, ElastPQ spleen stiffness and LSPS to predict the presence of high-risk oesophageal varices in PBC.*

Comparison of ElastPQ spleen stiffness with the Baveno VI criteria.

Finally, the performance of ElastPQ spleen stiffness in predicting CSPH was evaluated in comparison with the Baveno VI and Expanded Baveno VI criteria [228, 233]. This analysis was performed only on 40 patients who had an OGD within a year from the elastographic assessment and a valid spleen stiffness measurement. Among these, 9 patients were found to have OVs. Both Baveno VI and Expanded Baveno VI criteria showed a low sensitivity, therefore a low PPV for OVs (PPV 0.46 and 0.60 for Baveno VI and Expanded Baveno VI, respectively). In contrast, spleen stiffness had a good sensitivity (89%) and an excellent specificity (97%), with a best cut-off of 50.1 kPa. Using spleen stiffness as a predictor of CSPH, the rate of both false positive and false negative results dropped significantly in comparison to the Baveno VI and the Expanded Baveno VI criteria, reaching the 0% when the quality criterion of ElastPQ spleen stiffness SD/mean ≤30% was applied.

The performance of the Baveno VI, Expanded Baveno VI and ElastPQ spleen stiffness to identify patients at high-risk to have OVs is summarized in **Table 29**. The misclassification of the patients with or without OVs at endoscopy and the number of the endoscopies that could have been saved using the Baveno VI criteria, the Expanded Baveno VI criteria or ElastPQ spleen stiffness (≥50.1 kPa or <50.1 kPa) are reported in **Table 30.**

Twenty-seven (66%) and 30 (75%) patients met the Baveno VI and the Expanded Baveno VI criteria for avoiding endoscopy. These would have saved 77% and 87% of the wasted OGDs, respectively. Thirty-one (76%) patients had ElastPQ spleen stiffness <50.1 kPa (low-risk). The rate of the saved unnecessary endoscopies using ElastPQ spleen stiffness to determine the need of a screening OGDs would have risen to 97%, reaching the 100% when criterion of ElastPQ spleen stiffness SD/mean ≤30%

was applied. Furthermore, the use of ElastPQ spleen stiffness as a discriminant of risk of CSPH reduced the number of the missed endoscopies compared to Baveno VI and the Expanded Baveno VI criteria, which performance was similar in this cohort (**Table 30**).

Table 29. Performance of Baveno VI criteria, Expanded Baveno VI criteria and spleen stiffness in predicting the presence of oesophageal varices in PBC.

	OVs/n	Cut-off	Se	Sp	PPV	NPV	PLR	NLR
Baveno VI	9/40	-	0.67	0.77	0.46	0.89	2.0	0.42
Expanded Baveno VI	9/40	-	0.67	0.87	0.60	0.90	5.15	0.37
ElastPQ spleen stiffness	9/40	50.1 kPa	0.89	0.97	0.89	0.97	29.7	0.11

Abbreviations: PBC primary biliary cholangitis; OVs, oesophageal varices; n, number of patients with varices; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

Table 30. Misclassification and performance of the patients with or without oesophageal varices at endoscopy using Baveno VI criteria, Expanded Baveno VI criteria and ElastPQ spleen stiffness.

	FN	FP	Spared OGDs*	Missed OVs**
Baveno VI	3	7	24/31 (77%)	3/27 (11%)
Baveno VI expanded	3	4	27/31 (87%)	3/30 (10%)
ElastPQ spleen stiffness	1	1	30/31 (97%)	1/31 (3%)
ElastPQ spleen stiffness SD/mean ≤30%	0	0	31/31 (100%)	0/23 (0%)

Abbreviations: FN, false negative; FP, false positive; OVs, oesophageal varices; OGDs, oesophagogastroscopies; SD, standard deviation.

** Spared OGDs are expressed as: (number of patients who did not have OVs at endoscopy – FP) / number of patients who did not have OVs at endoscopy %.*

** Missed OGDs are expressed as: FN / number of patients who met criteria for avoiding endoscopy %.*

ElastPQ spleen stiffness performed better than the Baveno VI and Expanded Baveno VI criteria also for detecting patients with HRVs (cut-off for high-risk 53.4 kPa). ElastPQ spleen stiffness reduced both the number of the number of false positive and false negative obtained with Baveno VI and Expanded Baveno VI criteria, reaching its higher performance when the quality criterion SD/mean ≤30% was applied (**Table 31**).

The misclassification of the patients with or without OVs at endoscopy using the three tests is reported in **Table 32.** However, numbers in this cohort are very small (only 4 patients with HRVs) and therefore these results are reported mainly for a descriptive purpose.

Table 31. Performance of Baveno VI criteria, Expanded Baveno VI criteria and ElastPQ spleen stiffness in predicting the presence of high-risk oesophageal varices in this cohort of PBC patients.

	HRVs/n	Cut-off	Se	Sp	PPV	NPV	PLR	NLR
Baveno VI	4/40	-	0.75	0.72	0.23	0.96	2.7	0.34
Expanded Baveno VI	4/40	-	0.75	0.81	0.30	0.97	3.9	0.31
ElastPQ spleen stiffness	4/40	54.3 kPa	1.0	0.89	0.50	1.0	9.1	0

Abbreviations: PBC primary biliary cholangitis; HRVs, high-risk oesophageal varices; n, number of patients with varices; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

Table 32. Misclassification and performance of the patients with or without high-risk oesophageal varices at endoscopy using Baveno VI criteria, Expanded Baveno VI criteria and ElastPQ spleen stiffness.

	FN	FP	Spared OGDs*	Missed OVs**
Baveno VI	1	10	26/36 (72%)	1/27 (3.7%)
Baveno VI expanded	1	7	29/36 (81%)	1/30 (3.3%)
ElastPQ spleen stiffness	0	5	31/36 (86%)	0/32 (0%)
ElastPQ spleen stiffness SD/mean ≤30%	0	0	26/26 (100%)	0/23 (0%)

Abbreviations: FN, false negative; FP, false positive; OVs, oesophageal varices; OGDs, oesophagogastroscopies; SD, standard deviation.

** Spared OGDs are expressed as: (number of patients who did not have HRVs at endoscopy – FP) / number of patients who did not have HRVs at endoscopy %.*

*** Missed OGDs are expressed as: FN / number of patients who met criteria for avoiding endoscopy %.*

5. Discussion

The present results demonstrate that ElastPQ pSWE is an accurate non-invasive tool for staging liver disease in chronic primary cholestatic liver disease. Both in PSC and PBC, liver point share wave elastography measured with the ElastPQ technique strongly correlated with F-TE and the currently used scores of liver fibrosis, as well as with the scores of disease severity. Furthermore, transient elastography and, in particular ElastPQ spleen stiffness, proved to be a useful tool for the non-invasive diagnosis of clinically significant portal hypertension in this group of patients.

To date, reports regarding pSWE in PSC and PBC are scarce, mainly consisting in small cases vs. controls studies or scattered patients in the context of multi-aetiology evaluations [276], with a single larger study on PBC cirrhotics vs. controls evaluating pSWE in comparison with serum fibrosis markers throughout the Child-Pugh scores. Furthermore, the majority of these studies used ARFI pSWE [17]. Therefore, this is the first evaluation of the performance of ElastPQ elastography in a large cohort of primary sclerosing cholangitis and primary biliary cholangitis.

Over the last decades there has been a growing interest in the research of non-invasive surrogates for the staging of liver disease and, in particular, large efforts have been made in the attempt to identify markers of clinically significant portal hypertension, which can be life-threatening when unrecognised and untreated.

The advances in diagnostic methods have increased the proportion of patients with chronic liver disease diagnosed in the compensated stage of cirrhosis, often before the development of the PH-related complications [273]. The correct stratification of

prognostic risk is fundamental to ensure that an adequate personalised diagnostic and therapeutic approach is provided to the patients, maximizing the benefits and reducing the risks. Furthermore, the evidence that an effective aetiological treatment can lead to a variable degree of fibrosis regression [215] has raised the need of flexible prognostic markers for longitudinal follow-up.

Liver biopsy is still the gold standard for the diagnosis and staging of fibrosis. However, it is not free from drawbacks and complications such as invasiveness, sampling error, poor repeatability, inter-/intra-observer variability and risk of severe complications [277-279].

Similarly, the use of HVPG, the most validated tool to assess the presence and severity of portal hypertension, is limited by narrow availability, costs, invasiveness, and chances of complications.

In this context, the recent development of elastographic techniques offers valuable alternatives for the non-invasive assessment of liver disease stage, allowing to spare invasive procedures often obtaining real-time/bed-side diagnosis [255].

F-TE has a well-established role in the non-invasive assessment of liver fibrosis from various aetiologies, including PSC and PBC [11, 13, 255], and is widely used in clinical practice.

ElastPQ pSWE is a newly developed ultrasound-based technique, which has some advantages over F-TE, including the opportunity of wider liver scanning and a broader applicability (i.e. in patients obese or with ascites) [280].

ElastPQ has been proven to be accurate for the staging liver fibrosis in studies performed using liver biopsy as the reference standard, and a high level of concordance between F-TE and ElastPQ has been demonstrated in cohorts including mainly patients with chronic viral hepatitis [248, 281, 282]. It has been recently

demonstrated that F-TE has a very good correlation with histological fibrosis both in PBC and in PSC [13, 14, 283].

Based on these evidences, in the absence of an adequate number of liver biopsies in our cohort, owing to the fact that liver biopsy is not a standard requirement in clinical practice for patients with chronic cholestatic liver disease, the performance of ElastPQ was evaluated using F-TE as a surrogate of liver fibrosis.

ElastPQ liver stiffness and F-TE showed a very high level of agreement in both the diseases, showing a better concordance in the lower fibrosis stages. In fact, the main factor associated with a difference of 2 kPa or more between F-TE and ElastPQ liver stiffness was elevated grade of liver fibrosis/cirrhosis, in accordance to previous reports [280].

ElastPQ liver stiffness had a very good performance in discriminating between the stages of fibrosis, with a best accuracy for detecting advanced fibrosis/cirrhosis. In both cohorts, the misclassification was lower for the higher stages, with rates comparable to what has been obtained in the comparison between ElastPQ liver stiffness and liver histology in a large cohort of patients with hepatitis C [284]. From a prognostic point of view, the most important endpoint in patients with chronic liver disease is the assessment of liver cirrhosis [237]. Noticeably, in this cohort, the overall rate of patients correctly staged as F=4 by liver ElastPQ was 89-90%.

Similarly to what reported for F-TE [15], ElastPQ liver stiffness was better in ruling out than in ruling in cirrhosis, as already shown from a meta-analysis on the argument [249], with a best cut-off of 12.1 kPa in PSC (AUROC 0.99, 100% Se, 89% Sp) and 13.3 kPa in PBC (AUROC 0.97, 96% Se, 93% Sp). An inverse trend was noticed for the lower stages of fibrosis, where ElastPQ had higher specificity and poorer

sensitivity. The best cut-offs obtained for the diagnosis of cirrhosis were lower than the equivalent cut-offs for F-TE, in line with the data from the literature [280].

Interestingly, the application of quality criterion (ElastPQ SD/mean ≤30%) did not influence significantly the test accuracy and its concordance with F-TE. This is possibly related to the patchy distribution of fibrosis in the livers affected by PSC and PBC, which leads to a consequent higher variability of the liver measurements.

Liver ElastPQ, as well as F-TE, showed to be strongly associated with non-disease-specific markers of liver fibrosis, (except for AST/ALT ratio) with the highest rho Spearman's coefficient found for APRI, GUCI and ELF score. Slightly smaller Spearman's coefficients were obtained in the PBC cohort.

In PSC, liver elastography was also significantly associated with established prognostic scores such as MELD, the Oxford-Amsterdam score and, in particular, with the Mayo risk score. In PBC, the association with the Mayo risk score was still highly significant but less strong, likely due to the fact that all the patients included in this cohort were well compensated, with only 7 patients in Child Pugh class B and no patients in Child Pugh class C.

Total bilirubin, ALP and transaminases levels were also significantly associated with liver elastography performed both with F-TE and ElastPQ. Due to the lack of liver biopsies, the histological grade of inflammation could not be evaluated, and this is one of the limitations of this study. However, in order to avoid potential confounding factors, patients with AIH flares or very high transaminases were not included in the analysis. In the two studies evaluating F-TE in comparison with histological features in PSC and PBC, performed by Corpechot et al. did not found correlation between liver stiffness measured by TE and histological necroinflammatory activity. Moreover, on a multivariate analysis including serological markers of inflammation

and cholestasis, the stage of liver fibrosis remained the only independent parameter associated with liver stiffness [13, 14]. Because obstructive cholestasis has been shown to influence the results of F-TE, extrahepatic cholestasis (i.e. the presence of dominant strictures) was chosen as an exclusion criterion in the present study. However, a degree of intrahepatic cholestasis is intrinsic in the nature of PSC and PBC, and raised ALP and bilirubin are the most characteristic biochemical findings in these diseases [1]. ALP and bilirubin levels have been associated with disease progression and outcomes in PSC and PBC, and this association underlines further the potential role of liver elastography as a good surrogate prognostic marker [285, 286]. A lower level of correlation was also found between the above-reported variables and Elast-PQ spleen stiffness, which conceptually reflects the development of portal hypertension [247].

It is well known that, as a consequence of the haemodynamic changes subsequent to the increase of the pressure in the portal system, the spleen undergoes morphologic and functional changes [287]. Splenomegaly is usually one of the indirect signs of the presence of portal hypertension, but its degree may vary among the different liver disease aetiologies. Spleen diameter has been reported to be an independent predictor of OVs in compensated cirrhosis (and has been included in combined scores to evaluate the risk of CSPH) [273], but it loses its predictivity in the more advanced stages of the disease [288], reflecting a nonlinear relationship with PH [245].

In both PSC and PBC cohort, spleen stiffness was significantly higher in patients with values of liver elastography compatible with advanced fibrosis and cirrhosis (stages F3-F4), compared to patients with no-to-moderate fibrosis (Stages F0-F2).

The correlation between spleen stiffness and portal hypertension as measured by HVPG has been reported, and a meta-analysis of 16 studies performed by different

ultrasound elastography techniques, concluded for a better performance of spleen stiffness, compared to liver stiffness, in predicting the presence of OVs [258]. However, a statistically significant difference was detected only with regards to the specificity, while no difference between LSM and SSM was found in terms of sensitivity. Also, this meta-analysis comprises data from studies including patients with decompensated cirrhosis who, by definition, have CSPH and therefore high likelihood of having OVs. Another meta-analysis could not conclude for a definitive utility of SSM as a reliable predictor of OVs presence, due to the significant heterogeneity among the studies included, in particular owing to differences in elastography techniques used, and the high risk of bias [289]. Hence, the superiority of SSM versus LSM for the diagnosis of PH in compensated advanced chronic liver disease has not been definitively proven [257].

To date, data regarding the diagnostic performance of spleen stiffness measured with FibroScan in predicting CSPH are quite controversial and characterized by limitations such as heterogeneous cut-offs according to liver disease aetiology, high rate of failure, in particular in small-sized spleens, and a ceiling value of 75 kPa, which limits risk stratification above this threshold [237, 245, 255]. Point-SWE overcomes most of these limitations and data on spleen stiffness assessment by ARFI are promising, although need further validation [290]. Data regarding ElastPQ, in this context are currently missing [247].

In the present study, ElastPQ spleen stiffness was the only independent factor of CSPH in both cohorts of patients (OR 1.14, 95%CI 1.02-1.27, P=0.021 in PSC; OR 1.14, 95%CI 1.01-1.28, P=0.033 in PBC). In the group of patients with PSC, F-TE, ElastPQ liver stiffness, ElastPQ spleen stiffness and LSPS performed better than the other non-invasive diagnostic tests in detecting the presence of OVs, with similar

AUROCs (between 0.87 and 0.89). All the tests had a very good sensitivity, while specificity ranged between 68% and 77%, implying a better performance in ruling out than in ruling in the presence of CSPH.

In the group of the PBC patients, instead, ElastPQ spleen stiffness showed a significantly better performance compared to liver stiffness (F-TE and ElastPQ), reaching an AUROC of 0.95, with a best cut-off of 50.1 kPa. ElastPQ spleen stiffness had a very good sensitivity (89%) and an excellent specificity (97%) for the diagnosis of CSPH. LSPS showed an AUROC of 90%, with a best cut-off of 1.2, comparable to what reported in the literature [291].

With a cut-off of 53.4 kPa, in the PBC cohort ElastPQ spleen stiffness was also the best predictor of the presence of HRVs (AURO 0.94, 95%CI 0.87-1.0; Se 100%, Sp 89%). However numbers were very small in this cohort, and these results need to be interpreted carefully.

The application of the Baveno VI criteria allows sparing between 10% to 30% of screening endoscopies in patients with compensated chronic liver disease, with a very low risk of missing HRVs that need treatment [231, 233, 234]. In a large cohort of patients with cirrhosis due to hepatitis C, alcoholic and non-alcoholic steatohepatitis, the Expanded Baveno VI criteria increased the number of spared endoscopies to 40%, while maintaining risk of missing HRVs below the accepted threshold of <5%. When specifically examined in a multicenter cohort of patient with chronic cholestatic liver disease, both the criteria showed a good applicability in PSC, while in PBC the Expanded criteria resulted in an unacceptable number of missed varices (6%) [235].

In the present study, both the Baveno VI and the Expanded Baveno VI criteria were characterised by a high sensitivity but a relatively low specificity for predicting the presence of OVs of any grade.

In the PSC group, ElastPQ spleen stiffness (with a best performance when the quality criterion of SD/mean ≤30% was applied) had a higher specificity and reduced the number of FN obtained with the two sets of criteria, leading to a significant increase of the endoscopies that could have been saved, without increasing the number of unscreened varices. ElastPQ spleen stiffness showed an excellent performance as a discriminant of risk of CSPH in PBC. In this cohort, stratifying patients according to ElastPQ spleen stiffness values would have saved up to 100% of the wasted screening endoscopies, also decreasing the number of the missed OVs compared to Baveno VI and the Expanded Baveno VI criteria.

The higher performance of SSM compared to LSM in PBC is in keeping with the pre-sinusoidal component of portal hypertension typical of PBC [291]. In these patients, the portal-to-portal distribution of fibrosis and the development of porto-portal septa lead to an increased presinusoidal resistance, with a consequent increase of the portal pressure that, because of its peculiar characteristic, is underestimated by HVPG measurement [215].

In summary, this study supports the utility of LSM by ElastPQ for staging liver fibrosis and disease severity, both in PSC and PBC. Additionally, and in contrast with some previous reports failing to demonstrate an adequate reliability of SSM by different methods to rule-out any OVs, these data provide evidence on the excellent diagnostic performance of SSM by ElastPQ for detecting CSPH, and in particular for excluding the presence of OVs of any grade**,** in this group of patients. In this regard, spleen ElastPQ was even superior to the validated Baveno VI recommendations, although these refer to the use of TE to rule-out HRVs.

Limitations of this study include the lack of histology as a standard reference for liver

fibrosis stage, the small number of patients with OGDs within 12 months, the small number of patients with OVs and, among these, the very small number of HRVs. For this reason, these results warrant validation in larger, independent cohorts.

6. Conclusions

This study provides evidence of the suitability of liver and spleen ElastPQ elastography as valuable diagnostic and prognostic markers in PSC and PBC.

Nevertheless, the combination of different non-invasive tests providing often complementary information remains the most reliable approach for improving patients' risk-stratification and reducing the risk of false-positive and false-negative results.

References

1. European Association for the Study of the L. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. J Hepatol. 2009;51:237-67.
2. Heathcote EJ. Diagnosis and management of cholestatic liver disease. Clin Gastroenterol Hepatol. 2007;5:776-82.
3. Karlsen TH, Vesterhus M, Boberg KM. Review article: controversies in the management of primary biliary cirrhosis and primary sclerosing cholangitis. Aliment Pharmacol Ther. 2014;39:282-301.
4. Ponsioen CY, Lindor KD, Mehta R, et al. Design and Endpoints for Clinical Trials in Primary Sclerosing Cholangitis. Hepatology. 2018.
5. Vesterhus M, Hov JR, Holm A, et al. Enhanced liver fibrosis score predicts transplant-free survival in primary sclerosing cholangitis. Hepatology. 2015;62:188-97.
6. de Vries EMG, Farkkila M, Milkiewicz P, et al. Enhanced liver fibrosis test predicts transplant-free survival in primary sclerosing cholangitis, a multi-centre study. Liver Int. 2017;37:1554-61.
7. Nielsen MJ, Thorburn D, Leeming DJ, et al. Serological markers of extracellular matrix remodeling predict transplant-free survival in primary sclerosing cholangitis. Aliment Pharmacol Ther. 2018;48:179-89.
8. Trivedi PJ, Tickle J, Vesterhus MN, et al. Vascular adhesion protein-1 is elevated in primary sclerosing cholangitis, is predictive of clinical outcome and facilitates recruitment of gut-tropic lymphocytes to liver in a substrate-dependent manner. Gut. 2018;67:1135-45.
9. Vesterhus M, Holm A, Hov JR, et al. Novel serum and bile protein markers predict primary sclerosing cholangitis disease severity and prognosis. J Hepatol. 2017;66:1214-22.
10. Kouroumalis E, Samonakis D, Voumvouraki A. Biomarkers for primary biliary cholangitis: current perspectives. Hepat Med. 2018;10:43-53.

11. Corpechot C, El Naggar A, Poujol-Robert A, et al. Assessment of biliary fibrosis by transient elastography in patients with PBC and PSC. Hepatology. 2006;43:1118-24.

12. Gomez-Dominguez E, Mendoza J, Garcia-Buey L, et al. Transient elastography to assess hepatic fibrosis in primary biliary cirrhosis. Aliment Pharmacol Ther. 2008;27:441-7.

13. Corpechot C, Carrat F, Poujol-Robert A, et al. Noninvasive elastography-based assessment of liver fibrosis progression and prognosis in primary biliary cirrhosis. Hepatology. 2012;56:198-208.

14. Corpechot C, Gaouar F, El Naggar A, et al. Baseline values and changes in liver stiffness measured by transient elastography are associated with severity of fibrosis and outcomes of patients with primary sclerosing cholangitis. Gastroenterology. 2014;146:970-9; quiz e15-6.

15. Ehlken H, Wroblewski R, Corpechot C, et al. Validation of Transient Elastography and Comparison with Spleen Length Measurement for Staging of Fibrosis and Clinical Prognosis in Primary Sclerosing Cholangitis. PLoS One. 2016;11:e0164224.

16. Eaton JE, Dzyubak B, Venkatesh SK, et al. Performance of magnetic resonance elastography in primary sclerosing cholangitis. J Gastroenterol Hepatol. 2016;31:1184-90.

17. Zhang HC, Hu RF, Zhu T, et al. Primary biliary cirrhosis degree assessment by acoustic radiation force impulse imaging and hepatic fibrosis indicators. World J Gastroenterol. 2016;22:5276-84.

18. Hirschfield GM, Karlsen TH, Lindor KD, et al. Primary sclerosing cholangitis. Lancet. 2013;382:1587-99.

19. Maggs JR, Chapman RW. An update on primary sclerosing cholangitis. Curr Opin Gastroenterol. 2008;24:377-83.

20. Tischendorf JJ, Hecker H, Kruger M, et al. Characterization, outcome, and prognosis in 273 patients with primary sclerosing cholangitis: A single center study. The American journal of gastroenterology. 2007;102:107-14.

21. Molodecky NA, Myers RP, Barkema HW, et al. Validity of administrative data for the diagnosis of primary sclerosing cholangitis: a population-based study. Liver Int. 2011;31:712-20.

22. Molodecky NA, Kareemi H, Parab R, et al. Incidence of primary sclerosing cholangitis: a systematic review and meta-analysis. Hepatology. 2011;53:1590-9.

23. Jepsen P, Gronbaek L, Vilstrup H. Worldwide Incidence of Autoimmune Liver Disease. Dig Dis. 2015;33 Suppl 2:2-12.

24. Boberg KM, Aadland E, Jahnsen J, et al. Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population. Scand J Gastroenterol. 1998;33:99-103.

25. Bambha K, Kim WR, Talwalkar J, et al. Incidence, clinical spectrum, and outcomes of primary sclerosing cholangitis in a United States community. Gastroenterology. 2003;125:1364-9.

26. Escorsell A, Pares A, Rodes J, et al. Epidemiology of primary sclerosing cholangitis in Spain. Spanish Association for the Study of the Liver. J Hepatol. 1994;21:787-91.

27. Lindkvist B, Benito de Valle M, Gullberg B, et al. Incidence and prevalence of primary sclerosing cholangitis in a defined adult population in Sweden. Hepatology. 2010;52:571-7.

28. Boonstra K, Weersma RK, van Erpecum KJ, et al. Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis. Hepatology. 2013;58:2045-55.

29. Weismuller TJ, Trivedi PJ, Bergquist A, et al. Patient Age, Sex, and Inflammatory Bowel Disease Phenotype Associate With Course of Primary Sclerosing Cholangitis. Gastroenterology. 2017;152:1975-84 e8.

30. Boonstra K, Beuers U, Ponsioen CY. Epidemiology of primary sclerosing cholangitis and primary biliary cirrhosis: a systematic review. J Hepatol. 2012;56:1181-8.

31. Riley TR, Schoen RE, Lee RG, et al. A case series of transplant recipients who despite immunosuppression developed inflammatory bowel disease. Am J Gastroenterol. 1997;92:279-82.

32. Schrumpf E, Elgjo K, Fausa O, et al. Sclerosing cholangitis in ulcerative colitis. Scand J Gastroenterol. 1980;15:689-97.

33. Befeler AS, Lissoos TW, Schiano TD, et al. Clinical course and management of inflammatory bowel disease after liver transplantation. Transplantation. 1998;65:393-6.

34. Halliday JS, Djordjevic J, Lust M, et al. A unique clinical phenotype of primary sclerosing cholangitis associated with Crohn's disease. J Crohns Colitis. 2012;6:174-81.

35. Saarinen S, Olerup O, Broome U. Increased frequency of autoimmune diseases in patients with primary sclerosing cholangitis. Am J Gastroenterol. 2000;95:3195-9.

36. Bjornsson E. Small-duct primary sclerosing cholangitis. Curr Gastroenterol Rep. 2009;11:37-41.

37. Karlsen TH, Boberg KM. Update on primary sclerosing cholangitis. J Hepatol. 2013;59:571-82.

38. Yimam KK, Bowlus CL. Diagnosis and classification of primary sclerosing cholangitis. Autoimmun Rev. 2014;13:445-50.

39. Webster GJ, Pereira SP, Chapman RW. Autoimmune pancreatitis/IgG4-associated cholangitis and primary sclerosing cholangitis--overlapping or separate diseases? J Hepatol. 2009;51:398-402.

40. Okazaki K, Uchida K, Koyabu M, et al. IgG4 cholangiopathy: current concept, diagnosis, and pathogenesis. J Hepatol. 2014;61:690-5.

41. Blechacz B. Cholangiocarcinoma: Current Knowledge and New Developments. Gut Liver. 2017;11:13-26.

42. Ehlken H, Zenouzi R, Schramm C. Risk of cholangiocarcinoma in patients with primary sclerosing cholangitis: diagnosis and surveillance. Curr Opin Gastroenterol. 2017;33:78-84.

43. Eaton JE, Talwalkar JA, Lazaridis KN, et al. Pathogenesis of primary sclerosing cholangitis and advances in diagnosis and management. Gastroenterology. 2013;145:521-36.

44. Zenouzi R, Weismuller TJ, Hubener P, et al. Low risk of hepatocellular carcinoma in patients with primary sclerosing cholangitis with cirrhosis. Clin Gastroenterol Hepatol. 2014;12:1733-8.

45. Gossard AA, Lindor KD. Hepatocellular carcinoma: low risk of HCC in patients who have PSC and cirrhosis. Nat Rev Gastroenterol Hepatol. 2014;11:276-7.

46. Said K, Glaumann H, Bergquist A. Gallbladder disease in patients with primary sclerosing cholangitis. J Hepatol. 2008;48:598-605.

47. Brandt DJ, MacCarty RL, Charboneau JW, et al. Gallbladder disease in patients with primary sclerosing cholangitis. AJR Am J Roentgenol. 1988;150:571-4.

48. Buckles DC, Lindor KD, Larusso NF, et al. In primary sclerosing cholangitis, gallbladder polyps are frequently malignant. Am J Gastroenterol. 2002;97:1138-42.

49. Lewis JT, Talwalkar JA, Rosen CB, et al. Prevalence and risk factors for gallbladder neoplasia in patients with primary sclerosing cholangitis: evidence for a metaplasia-dysplasia-carcinoma sequence. Am J Surg Pathol. 2007;31:907-13.

50. Gourevitch RA, Rose S, Crockett SD, et al. Variation in Pathologist Classification of Colorectal Adenomas and Serrated Polyps. Am J Gastroenterol. 2018;113:431-9.

51. Bergquist A, Ekbom A, Olsson R, et al. Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. J Hepatol. 2002;36:321-7.

52. Soetikno RM, Lin OS, Heidenreich PA, et al. Increased risk of colorectal neoplasia in patients with primary sclerosing cholangitis and ulcerative colitis: a meta-analysis. Gastrointest Endosc. 2002;56:48-54.

53. Liu JZ, Hov JR, Folseraas T, et al. Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis. Nat Genet. 2013;45:670-5.

54. Karlsen TH. A lecture on the genetics of primary sclerosing cholangitis. Dig Dis. 2012;30 Suppl 1:32-8.

55. Karlsen TH, Kaser A. Deciphering the genetic predisposition to primary sclerosing cholangitis. Semin Liver Dis. 2011;31:188-207.

56. Folseraas T, Melum E, Franke A, et al. Genetics in primary sclerosing cholangitis. Best Pract Res Clin Gastroenterol. 2011;25:713-26.

57. Naess S, Shiryaev A, Hov JR, et al. Genetics in primary sclerosing cholangitis. Clin Res Hepatol Gastroenterol. 2012;36:325-33.

58. Ji SG, Juran BD, Mucha S, et al. Genome-wide association study of primary sclerosing cholangitis identifies new risk loci and quantifies the genetic relationship with inflammatory bowel disease. Nat Genet. 2017;49:269-73.

59. Ellinghaus D, Jostins L, Spain SL, et al. Analysis of five chronic inflammatory diseases identifies 27 new associations and highlights disease-specific patterns at shared loci. Nat Genet. 2016;48:510-8.

60. Karlsen TH, Folseraas T, Thorburn D, et al. Primary sclerosing cholangitis - a comprehensive review. J Hepatol. 2017;67:1298-323.

61. Hov JR, Karlsen TH. The Microbiome in Primary Sclerosing Cholangitis: Current Evidence and Potential Concepts. Semin Liver Dis. 2017;37:314-31.

62. Adams DH, Eksteen B. Aberrant homing of mucosal T cells and extra-intestinal manifestations of inflammatory bowel disease. Nat Rev Immunol. 2006;6:244-51.

63. Grant AJ, Lalor PF, Hubscher SG, et al. MAdCAM-1 expressed in chronic inflammatory liver disease supports mucosal lymphocyte adhesion to hepatic endothelium (MAdCAM-1 in chronic inflammatory liver disease). Hepatology. 2001;33:1065-72.

64. Eksteen B, Grant AJ, Miles A, et al. Hepatic endothelial CCL25 mediates the recruitment of CCR9+ gut-homing lymphocytes to the liver in primary sclerosing cholangitis. J Exp Med. 2004;200:1511-7.

65. O'Hara SP, Karlsen TH, LaRusso NF. Cholangiocytes and the environment in primary sclerosing cholangitis: where is the link? Gut. 2017;66:1873-7.

66. Kamihira T, Shimoda S, Nakamura M, et al. Biliary epithelial cells regulate autoreactive T cells: implications for biliary-specific diseases. Hepatology. 2005;41:151-9.

67. Xu B, Broome U, Ericzon BG, et al. High frequency of autoantibodies in patients with primary sclerosing cholangitis that bind biliary epithelial cells and induce expression of CD44 and production of interleukin 6. Gut. 2002;51:120-7.

68. Bottcher K, Rombouts K, Saffioti F, et al. MAIT cells are chronically activated in patients with autoimmune liver disease and promote profibrogenic hepatic stellate cell activation. Hepatology. 2018;68:172-86.

69. Hov JR, Boberg KM, Karlsen TH. Autoantibodies in primary sclerosing cholangitis. World J Gastroenterol. 2008;14:3781-91.

70. Terjung B, Sohne J, Lechtenberg B, et al. p-ANCAs in autoimmune liver disorders recognise human beta-tubulin isotype 5 and cross-react with microbial protein FtsZ. Gut. 2010;59:808-16.

71. Trauner M, Meier PJ, Boyer JL. Molecular pathogenesis of cholestasis. N Engl J Med. 1998;339:1217-27.

72. Dutta AK, Khimji AK, Kresge C, et al. Identification and functional characterization of TMEM16A, a Ca2+-activated Cl- channel activated by extracellular nucleotides, in biliary epithelium. J Biol Chem. 2011;286:766-76.

73. Broome U, Olsson R, Loof L, et al. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. Gut. 1996;38:610-5.

74. Saffioti F, Gurusamy KS, Hawkins N, et al. Pharmacological interventions for primary sclerosing cholangitis: an attempted network meta-analysis. Cochrane Database Syst Rev. 2017;3:CD011343.

75. Kotlyar DS, Campbell MS, Reddy KR. Recurrence of diseases following orthotopic liver transplantation. Am J Gastroenterol. 2006;101:1370-8.

76. Ravikumar R, Tsochatzis E, Jose S, et al. Risk factors for recurrent primary sclerosing cholangitis after liver transplantation. J Hepatol. 2015;63:1139-46.

77. Chapman R, Fevery J, Kalloo A, et al. Diagnosis and management of primary sclerosing cholangitis. Hepatology. 2010;51:660-78.

78. Portmann B, Zen Y. Inflammatory disease of the bile ducts-cholangiopathies: liver biopsy challenge and clinicopathological correlation. Histopathology. 2012;60:236-48.

79. Nakanuma Y, Harada K, Katayanagi K, et al. Definition and pathology of primary sclerosing cholangitis. J Hepatobiliary Pancreat Surg. 1999;6:333-42.

80. Vitellas KM, Keogan MT, Freed KS, et al. Radiologic manifestations of sclerosing cholangitis with emphasis on MR cholangiopancreatography. Radiographics. 2000;20:959-75; quiz 1108-9, 12.

81. Wiesner RH, Grambsch PM, Dickson ER, et al. Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. Hepatology. 1989;10:430-6.

82. Farrant JM, Hayllar KM, Wilkinson ML, et al. Natural history and prognostic variables in primary sclerosing cholangitis. Gastroenterology. 1991;100:1710-7.

83. Dickson ER, Murtaugh PA, Wiesner RH, et al. Primary sclerosing cholangitis: refinement and validation of survival models. Gastroenterology. 1992;103:1893-901.

84. Kim WR, Therneau TM, Wiesner RH, et al. A revised natural history model for primary sclerosing cholangitis. Mayo Clin Proc. 2000;75:688-94.

85. de Vries EM, Wang J, Williamson KD, et al. A novel prognostic model for transplant-free survival in primary sclerosing cholangitis. Gut. 2018;67:1864-9.

86. Craig DA, MacCarty RL, Wiesner RH, et al. Primary sclerosing cholangitis: value of cholangiography in determining the prognosis. AJR Am J Roentgenol. 1991;157:959-64.

87. Ponsioen CY, Vrouenraets SM, Prawirodirdjo W, et al. Natural history of primary sclerosing cholangitis and prognostic value of cholangiography in a Dutch population. Gut. 2002;51:562-6.

88. Ruiz A, Lemoinne S, Carrat F, et al. Radiologic course of primary sclerosing cholangitis: assessment by three-dimensional magnetic resonance cholangiography and predictive features of progression. Hepatology. 2014;59:242-50.

89. Mitchell SA, Bansi DS, Hunt N, et al. A preliminary trial of high-dose ursodeoxycholic acid in primary sclerosing cholangitis. Gastroenterology. 2001;121:900-7.

90. Angulo P, Batts KP, Jorgensen RA, et al. Oral budesonide in the treatment of primary sclerosing cholangitis. Am J Gastroenterol. 2000;95:2333-7.

91. Angulo P, Larson DR, Therneau TM, et al. Time course of histological progression in primary sclerosing cholangitis. Am J Gastroenterol. 1999;94:3310-3.

92. de Vries EM, Verheij J, Hubscher SG, et al. Applicability and prognostic value of histologic scoring systems in primary sclerosing cholangitis. J Hepatol. 2015;63:1212-9.

93. de Vries EM, de Krijger M, Farkkila M, et al. Validation of the prognostic value of histologic scoring systems in primary sclerosing cholangitis: An international cohort study. Hepatology. 2017;65:907-19.

94. Calvaruso V, Burroughs AK, Standish R, et al. Computer-assisted image analysis of liver collagen: relationship to Ishak scoring and hepatic venous pressure gradient. Hepatology. 2009;49:1236-44.

95. Standish RA, Cholongitas E, Dhillon A, et al. An appraisal of the histopathological assessment of liver fibrosis. Gut. 2006;55:569-78.

96. Olsson R, Boberg KM, de Muckadell OS, et al. High-dose ursodeoxycholic acid in primary sclerosing cholangitis: a 5-year multicenter, randomized, controlled study. Gastroenterology. 2005;129:1464-72.

97. Lindor KD, Kowdley KV, Luketic VA, et al. High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. Hepatology. 2009;50:808-14.

98. Stanich PP, Bjornsson E, Gossard AA, et al. Alkaline phosphatase normalization is associated with better prognosis in primary sclerosing cholangitis. Dig Liver Dis. 2011;43:309-13.

99. Lindstrom L, Hultcrantz R, Boberg KM, et al. Association between reduced levels of alkaline phosphatase and survival times of patients with primary sclerosing cholangitis. Clin Gastroenterol Hepatol. 2013;11:841-6.

100. Rupp C, Rossler A, Halibasic E, et al. Reduction in alkaline phosphatase is associated with longer survival in primary sclerosing cholangitis, independent of dominant stenosis. Aliment Pharmacol Ther. 2014;40:1292-301.

101. Al Mamari S, Djordjevic J, Halliday JS, et al. Improvement of serum alkaline phosphatase to <1.5 upper limit of normal predicts better outcome and reduced risk of cholangiocarcinoma in primary sclerosing cholangitis. J Hepatol. 2013;58:329-34.

102. Talwalkar JA, Chapman RW. The resurgence of serum alkaline phosphatase as a surrogate biomarker for prognosis in primary sclerosing cholangitis. Clin Gastroenterol Hepatol. 2013;11:847-9.

103. Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence of liver fibrosis: a cohort study. Gastroenterology. 2004;127:1704-13.

104. Beuers U, Gershwin ME, Gish RG, et al. Changing nomenclature for PBC: From 'cirrhosis' to 'cholangitis'. J Hepatol. 2015;63:1285-7.

105. Moteki S, Leung PS, Dickson ER, et al. Epitope mapping and reactivity of autoantibodies to the E2 component of 2-oxoglutarate dehydrogenase complex in primary biliary cirrhosis using recombinant 2-oxoglutarate dehydrogenase complex. Hepatology. 1996;23:436-44.

106. Brahmer JR, Rodriguez-Abreu D, Robinson AG, et al. Health-related quality-of-life results for pembrolizumab versus chemotherapy in advanced, PD-L1-positive NSCLC (KEYNOTE-024): a multicentre, international, randomised, open-label phase 3 trial. Lancet Oncol. 2017;18:1600-9.

107. Webb GJ, Hirschfield GM. Primary biliary cholangitis in 2016: High-definition PBC: biology, models and therapeutic advances. Nat Rev Gastroenterol Hepatol. 2017;14:76-8.

108. European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. J Hepatol. 2017;67:145-72.

109. Mells GF, Pells G, Newton JL, et al. Impact of primary biliary cirrhosis on perceived quality of life: the UK-PBC national study. Hepatology. 2013;58:273-83.

110. Dyson JK, Wilkinson N, Jopson L, et al. The inter-relationship of symptom severity and quality of life in 2055 patients with primary biliary cholangitis. Aliment Pharmacol Ther. 2016;44:1039-50.

111. Tsochatzis EA, Gurusamy KS, Gluud C, et al. Ursodeoxycholic acid and primary biliary cirrhosis: EASL and AASLD guidelines. J Hepatol. 2009;51:1084-5; author reply 5-6.

112. Lammert C, Juran BD, Schlicht E, et al. Biochemical response to ursodeoxycholic acid predicts survival in a North American cohort of primary biliary cirrhosis patients. J Gastroenterol. 2014;49:1414-20.

113. Corpechot C, Abenavoli L, Rabahi N, et al. Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. Hepatology. 2008;48:871-7.

114. Kumagi T, Guindi M, Fischer SE, et al. Baseline ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis. Am J Gastroenterol. 2010;105:2186-94.

115. Pares A, Caballeria L, Rodes J. Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic Acid. Gastroenterology. 2006;130:715-20.

116. Saffioti F, Gurusamy KS, Eusebi LH, et al. Pharmacological interventions for primary biliary cholangitis: an attempted network meta-analysis. Cochrane Database Syst Rev. 2017;3:CD011648.

117. Hirschfield GM, Mason A, Luketic V, et al. Efficacy of obeticholic acid in patients with primary biliary cirrhosis and inadequate response to ursodeoxycholic acid. Gastroenterology. 2015;148:751-61 e8.

118. Nevens F, Andreone P, Mazzella G, et al. A Placebo-Controlled Trial of Obeticholic Acid in Primary Biliary Cholangitis. N Engl J Med. 2016;375:631-43.

119. Lu M, Li J, Haller IV, et al. Factors Associated With Prevalence and Treatment of Primary Biliary Cholangitis in United States Health Systems. Clin Gastroenterol Hepatol. 2018;16:1333-41 e6.

120. Lleo A, Jepsen P, Morenghi E, et al. Evolving Trends in Female to Male Incidence and Male Mortality of Primary Biliary Cholangitis. Sci Rep. 2016;6:25906.

121. Selmi C, Bowlus CL, Gershwin ME, et al. Primary biliary cirrhosis. Lancet. 2011;377:1600-9.

122. Boonstra K, Kunst AE, Stadhouders PH, et al. Rising incidence and prevalence of primary biliary cirrhosis: a large population-based study. Liver Int. 2014;34:e31-8.

123. Griffiths L, Dyson JK, Jones DE. The new epidemiology of primary biliary cirrhosis. Semin Liver Dis. 2014;34:318-28.

124. Dahlqvist G, Gaouar F, Carrat F, et al. Large-scale characterization study of patients with antimitochondrial antibodies but nonestablished primary biliary cholangitis. Hepatology. 2017;65:152-63.

125. Inoue K, Hirohara J, Nakano T, et al. Prediction of prognosis of primary biliary cirrhosis in Japan. Liver. 1995;15:70-7.

126. Lleo A, Marzorati S, Anaya JM, et al. Primary biliary cholangitis: a comprehensive overview. Hepatol Int. 2017;11:485-99.

127. Mattalia A, Quaranta S, Leung PS, et al. Characterization of antimitochondrial antibodies in health adults. Hepatology. 1998;27:656-61.

128. Shibata M, Onozuka Y, Morizane T, et al. Prevalence of antimitochondrial antibody in Japanese corporate workers in Kanagawa prefecture. J Gastroenterol. 2004;39:255-9.

129. Metcalf JV, Mitchison HC, Palmer JM, et al. Natural history of early primary biliary cirrhosis. Lancet. 1996;348:1399-402.

130. Lazaridis KN, Juran BD, Boe GM, et al. Increased prevalence of antimitochondrial antibodies in first-degree relatives of patients with primary biliary cirrhosis. Hepatology. 2007;46:785-92.

131. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. N Engl J Med. 2005;353:1261-73.

132. Nakamura M, Kondo H, Mori T, et al. Anti-gp210 and anti-centromere antibodies are different risk factors for the progression of primary biliary cirrhosis. Hepatology. 2007;45:118-27.

133. Invernizzi P, Podda M, Battezzati PM, et al. Autoantibodies against nuclear pore complexes are associated with more active and severe liver disease in primary biliary cirrhosis. J Hepatol. 2001;34:366-72.

134. Taylor SL, Dean PJ, Riely CA. Primary autoimmune cholangitis. An alternative to antimitochondrial antibody-negative primary biliary cirrhosis. Am J Surg Pathol. 1994;18:91-9.

135. Invernizzi P, Crosignani A, Battezzati PM, et al. Comparison of the clinical features and clinical course of antimitochondrial antibody-positive and -negative primary biliary cirrhosis. Hepatology. 1997;25:1090-5.

136. Sakauchi F, Mori M, Zeniya M, et al. Antimitochondrial antibody negative primary biliary cirrhosis in Japan: utilization of clinical data when patients applied to receive public financial aid. J Epidemiol. 2006;16:30-4.

137. Lleo A, Liao J, Invernizzi P, et al. Immunoglobulin M levels inversely correlate with CD40 ligand promoter methylation in patients with primary biliary cirrhosis. Hepatology. 2012;55:153-60.

138. Longo M, Crosignani A, Battezzati PM, et al. Hyperlipidaemic state and cardiovascular risk in primary biliary cirrhosis. Gut. 2002;51:265-9.

139. Floreani A, Franceschet I, Cazzagon N, et al. Extrahepatic autoimmune conditions associated with primary biliary cirrhosis. Clin Rev Allergy Immunol. 2015;48:192-7.

140. Boberg KM, Chapman RW, Hirschfield GM, et al. Overlap syndromes: the International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. J Hepatol. 2011;54:374-85.

141. Selmi C, Mayo MJ, Bach N, et al. Primary biliary cirrhosis in monozygotic and dizygotic twins: genetics, epigenetics, and environment. Gastroenterology. 2004;127:485-92.

142. Shin S, Moh IH, Woo YS, et al. Evidence from a familial case suggests maternal inheritance of primary biliary cholangitis. World J Gastroenterol. 2017;23:7191-7.

143. Cheung AC, LaRusso NF, Gores GJ, et al. Epigenetics in the Primary Biliary Cholangitis and Primary Sclerosing Cholangitis. Semin Liver Dis. 2017;37:159-74.

144. Hirschfield GM, Gershwin ME. The immunobiology and pathophysiology of primary biliary cirrhosis. Annu Rev Pathol. 2013;8:303-30.

145. Lammert C, Nguyen DL, Juran BD, et al. Questionnaire based assessment of risk factors for primary biliary cirrhosis. Dig Liver Dis. 2013;45:589-94.

146. Prince MI, Ducker SJ, James OF. Case-control studies of risk factors for primary biliary cirrhosis in two United Kingdom populations. Gut. 2010;59:508-12.

147. Gershwin ME, Selmi C, Worman HJ, et al. Risk factors and comorbidities in primary biliary cirrhosis: a controlled interview-based study of 1032 patients. Hepatology. 2005;42:1194-202.

148. Invernizzi P, Ransom M, Raychaudhuri S, et al. Classical HLA-DRB1 and DPB1 alleles account for HLA associations with primary biliary cirrhosis. Genes Immun. 2012;13:461-8.

149. Liu JZ, Almarri MA, Gaffney DJ, et al. Dense fine-mapping study identifies new susceptibility loci for primary biliary cirrhosis. Nat Genet. 2012;44:1137-41.

150. Hirschfield GM, Liu X, Xu C, et al. Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. N Engl J Med. 2009;360:2544-55.

151. Cordell HJ, Han Y, Mells GF, et al. International genome-wide meta-analysis identifies new primary biliary cirrhosis risk loci and targetable pathogenic pathways. Nat Commun. 2015;6:8019.

152. Mells GF, Floyd JA, Morley KI, et al. Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. Nat Genet. 2011;43:329-32.

153. Liu X, Invernizzi P, Lu Y, et al. Genome-wide meta-analyses identify three loci associated with primary biliary cirrhosis. Nat Genet. 2010;42:658-60.

154. Qiu F, Tang R, Zuo X, et al. A genome-wide association study identifies six novel risk loci for primary biliary cholangitis. Nat Commun. 2017;8:14828.

155. Abdel-Rahman OM, Elsayed Z. Yttrium-90 microsphere radioembolisation for unresectable hepatocellular carcinoma. Cochrane Database Syst Rev. 2016;2:CD011313.

156. Nakamura M, Nishida N, Kawashima M, et al. Genome-wide association study identifies TNFSF15 and POU2AF1 as susceptibility loci for primary

biliary cirrhosis in the Japanese population. Am J Hum Genet. 2012;91:721-8.

157. Selmi C, Cavaciocchi F, Lleo A, et al. Genome-wide analysis of DNA methylation, copy number variation, and gene expression in monozygotic twins discordant for primary biliary cirrhosis. Front Immunol. 2014;5:128.

158. Invernizzi P, Miozzo M, Battezzati PM, et al. Frequency of monosomy X in women with primary biliary cirrhosis. Lancet. 2004;363:533-5.

159. Prothero KE, Stahl JM, Carrel L. Dosage compensation and gene expression on the mammalian X chromosome: one plus one does not always equal two. Chromosome Res. 2009;17:637-48.

160. Miozzo M, Selmi C, Gentilin B, et al. Preferential X chromosome loss but random inactivation characterize primary biliary cirrhosis. Hepatology. 2007;46:456-62.

161. Lleo A, Selmi C, Invernizzi P, et al. Apotopes and the biliary specificity of primary biliary cirrhosis. Hepatology. 2009;49:871-9.

162. Shimoda S, Nakamura M, Ishibashi H, et al. Molecular mimicry of mitochondrial and nuclear autoantigens in primary biliary cirrhosis. Gastroenterology. 2003;124:1915-25.

163. Selmi C, De Santis M, Cavaciocchi F, et al. Infectious agents and xenobiotics in the etiology of primary biliary cirrhosis. Dis Markers. 2010;29:287-99.

164. Beuers U, Hohenester S, de Buy Wenniger LJ, et al. The biliary HCO(3)(-) umbrella: a unifying hypothesis on pathogenetic and therapeutic aspects of fibrosing cholangiopathies. Hepatology. 2010;52:1489-96.

165. Rodrigues PM, Perugorria MJ, Santos-Laso A, et al. Primary biliary cholangitis: a tale of epigenetically-induced secretory failure? J Hepatol. 2018.

166. Hirschfield GM. Diagnosis of primary biliary cirrhosis. Best Pract Res Clin Gastroenterol. 2011;25:701-12.

167. Lindor KD, Gershwin ME, Poupon R, et al. Primary biliary cirrhosis. Hepatology. 2009;50:291-308.

168. Vergani D, Alvarez F, Bianchi FB, et al. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. J Hepatol. 2004;41:677-83.

169. Invernizzi P, Lleo A, Podda M. Interpreting serological tests in diagnosing autoimmune liver diseases. Semin Liver Dis. 2007;27:161-72.

170. Hirschfield GM, Heathcote EJ. Antimitochondrial antibody-negative primary biliary cirrhosis. Clin Liver Dis. 2008;12:323-31; viii-ix.

171. Jones DE, Metcalf JV, Collier JD, et al. Hepatocellular carcinoma in primary biliary cirrhosis and its impact on outcomes. Hepatology. 1997;26:1138-42.

172. Trivedi PJ, Lammers WJ, van Buuren HR, et al. Stratification of hepatocellular carcinoma risk in primary biliary cirrhosis: a multicentre international study. Gut. 2016;65:321-9.

173. Carbone M, Mells GF, Pells G, et al. Sex and age are determinants of the clinical phenotype of primary biliary cirrhosis and response to ursodeoxycholic acid. Gastroenterology. 2013;144:560-9 e7; quiz e13-4.

174. Mahl TC, Shockcor W, Boyer JL. Primary biliary cirrhosis: survival of a large cohort of symptomatic and asymptomatic patients followed for 24 years. J Hepatol. 1994;20:707-13.

175. Prince M, Chetwynd A, Newman W, et al. Survival and symptom progression in a geographically based cohort of patients with primary biliary cirrhosis: follow-up for up to 28 years. Gastroenterology. 2002;123:1044-51.

176. Springer J, Cauch-Dudek K, O'Rourke K, et al. Asymptomatic primary biliary cirrhosis: a study of its natural history and prognosis. Am J Gastroenterol. 1999;94:47-53.

177. Roll J, Boyer JL, Barry D, et al. The prognostic importance of clinical and histologic features in asymptomatic and symptomatic primary biliary cirrhosis. N Engl J Med. 1983;308:1-7.

178. Martin P, DiMartini A, Feng S, et al. Evaluation for liver transplantation in adults: 2013 practice guideline by the American Association for the Study

of Liver Diseases and the American Society of Transplantation. Hepatology. 2014;59:1144-65.

179. European Association for the Study of the Liver. Electronic address eee. EASL Clinical Practice Guidelines: Liver transplantation. J Hepatol. 2016;64:433-85.

180. Adam R, Karam V, Delvart V, et al. Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). J Hepatol. 2012;57:675-88.

181. Singal AK, Guturu P, Hmoud B, et al. Evolving frequency and outcomes of liver transplantation based on etiology of liver disease. Transplantation. 2013;95:755-60.

182. Carbone M, Neuberger JM. Autoimmune liver disease, autoimmunity and liver transplantation. J Hepatol. 2014;60:210-23.

183. Silveira MG, Talwalkar JA, Lindor KD, et al. Recurrent primary biliary cirrhosis after liver transplantation. Am J Transplant. 2010;10:720-6.

184. Bosch A, Dumortier J, Maucort-Boulch D, et al. Preventive administration of UDCA after liver transplantation for primary biliary cirrhosis is associated with a lower risk of disease recurrence. J Hepatol. 2015;63:1449-58.

185. MacQuillan GC, Neuberger J. Liver transplantation for primary biliary cirrhosis. Clin Liver Dis. 2003;7:941-56, ix.

186. Lindor KD, Therneau TM, Jorgensen RA, et al. Effects of ursodeoxycholic acid on survival in patients with primary biliary cirrhosis. Gastroenterology. 1996;110:1515-8.

187. Poupon RE, Poupon R, Balkau B. Ursodiol for the long-term treatment of primary biliary cirrhosis. The UDCA-PBC Study Group. N Engl J Med. 1994;330:1342-7.

188. Goulis J, Leandro G, Burroughs AK. Randomised controlled trials of ursodeoxycholic-acid therapy for primary biliary cirrhosis: a meta-analysis. Lancet. 1999;354:1053-60.

189. Reig A, Sese P, Pares A. Effects of Bezafibrate on Outcome and Pruritus in Primary Biliary Cholangitis With Suboptimal Ursodeoxycholic Acid Response. Am J Gastroenterol. 2018;113:49-55.

190. Quarneti C, Muratori P, Lalanne C, et al. Fatigue and pruritus at onset identify a more aggressive subset of primary biliary cirrhosis. Liver Int. 2015;35:636-41.

191. Jones DE, Al-Rifai A, Frith J, et al. Corrigendum to "The independent effects of fatigue and UDCA therapy on mortality in primary biliary cirrhosis: Results of a 9 year cohort follow-up" J. Hepatol. 2010 Nov; 53(5):911-917. J Hepatol. 2011;55:737.

192. Muratori P, Muratori L, Ferrari R, et al. Characterization and clinical impact of antinuclear antibodies in primary biliary cirrhosis. Am J Gastroenterol. 2003;98:431-7.

193. Rigopoulou EI, Davies ET, Pares A, et al. Prevalence and clinical significance of isotype specific antinuclear antibodies in primary biliary cirrhosis. Gut. 2005;54:528-32.

194. Wesierska-Gadek J, Penner E, Battezzati PM, et al. Correlation of initial autoantibody profile and clinical outcome in primary biliary cirrhosis. Hepatology. 2006;43:1135-44.

195. Nakamura M, Kondo H, Tanaka A, et al. Autoantibody status and histological variables influence biochemical response to treatment and long-term outcomes in Japanese patients with primary biliary cirrhosis. Hepatol Res. 2015;45:846-55.

196. Taha-Abdelaziz K, Hodgins DC, Alkie TN, et al. Oral administration of PLGA-encapsulated CpG ODN and Campylobacter jejuni lysate reduces cecal colonization by Campylobacter jejuni in chickens. Vaccine. 2018;36:388-94.

197. Kashihara H, Shimada M, Yoshikawa K, et al. Duodenal-jejunal bypass changes the composition of the gut microbiota. Surg Today. 2017;47:137-40.

198. ter Borg PC, Schalm SW, Hansen BE, et al. Prognosis of ursodeoxycholic Acid-treated patients with primary biliary cirrhosis. Results of a 10-yr cohort study involving 297 patients. Am J Gastroenterol. 2006;101:2044-50.

199. Dickson ER, Grambsch PM, Fleming TR, et al. Prognosis in primary biliary cirrhosis: model for decision making. Hepatology. 1989;10:1-7.

200. Malinchoc M, Kamath PS, Gordon FD, et al. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. Hepatology. 2000;31:864-71.

201. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histol. 1978;379:103-12.

202. Scheuer PJ. Primary biliary cirrhosis: diagnosis, pathology and pathogenesis. Postgrad Med J. 1983;59 Suppl 4:106-15.

203. Nakanuma Y, Zen Y, Harada K, et al. Application of a new histological staging and grading system for primary biliary cirrhosis to liver biopsy specimens: Interobserver agreement. Pathol Int. 2010;60:167-74.

204. Garrido MC, Hubscher SG. Accuracy of staging in primary biliary cirrhosis. J Clin Pathol. 1996;49:556-9.

205. Huet PM, Vincent C, Deslaurier J, et al. Portal hypertension and primary biliary cirrhosis: effect of long-term ursodeoxycholic acid treatment. Gastroenterology. 2008;135:1552-60.

206. Floreani A, Cazzagon N, Martines D, et al. Performance and utility of transient elastography and noninvasive markers of liver fibrosis in primary biliary cirrhosis. Dig Liver Dis. 2011;43:887-92.

207. Mayo MJ, Parkes J, Adams-Huet B, et al. Prediction of clinical outcomes in primary biliary cirrhosis by serum enhanced liver fibrosis assay. Hepatology. 2008;48:1549-57.

208. Friedrich-Rust M, Rosenberg W, Parkes J, et al. Comparison of ELF, FibroTest and FibroScan for the non-invasive assessment of liver fibrosis. BMC Gastroenterol. 2010;10:103.

209. Trivedi PJ, Bruns T, Cheung A, et al. Optimising risk stratification in primary biliary cirrhosis: AST/platelet ratio index predicts outcome independent of ursodeoxycholic acid response. J Hepatol. 2014;60:1249-58.

210. Carbone M, Nardi A, Flack S, et al. Pretreatment prediction of response to ursodeoxycholic acid in primary biliary cholangitis: development and validation of the UDCA Response Score. Lancet Gastroenterol Hepatol. 2018;3:626-34.

211. Camilleri M, Lembo A, Katzka DA. Opioids in Gastroenterology: Treating Adverse Effects and Creating Therapeutic Benefits. Clin Gastroenterol Hepatol. 2017;15:1338-49.

212. Carbone M, Sharp SJ, Flack S, et al. The UK-PBC risk scores: Derivation and validation of a scoring system for long-term prediction of end-stage liver disease in primary biliary cholangitis. Hepatology. 2016;63:930-50.

213. Lammers WJ, Hirschfield GM, Corpechot C, et al. Development and Validation of a Scoring System to Predict Outcomes of Patients With Primary Biliary Cirrhosis Receiving Ursodeoxycholic Acid Therapy. Gastroenterology. 2015;149:1804-12 e4.

214. Friedman SL, Bansal MB. Reversal of hepatic fibrosis -- fact or fantasy? Hepatology. 2006;43:S82-8.

215. Saffioti F, Pinzani M. Development and Regression of Cirrhosis. Dig Dis. 2016;34:374-81.

216. Pellicoro A, Ramachandran P, Iredale JP, et al. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. Nat Rev Immunol. 2014;14:181-94.

217. European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL Clinical Practice

Guidelines for the management of patients with decompensated cirrhosis. J Hepatol. 2018;69:406-60.

218. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. J Hepatol. 2006;44:217-31.

219. Iwakiri Y, Groszmann RJ. Vascular endothelial dysfunction in cirrhosis. J Hepatol. 2007;46:927-34.

220. Burroughs AK, Thalheimer U. Hepatic venous pressure gradient in 2010: optimal measurement is key. Hepatology. 2010;51:1894-6.

221. Groszmann RJ, Garcia-Tsao G, Bosch J, et al. Beta-blockers to prevent gastroesophageal varices in patients with cirrhosis. N Engl J Med. 2005;353:2254-61.

222. Ripoll C, Groszmann R, Garcia-Tsao G, et al. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. Gastroenterology. 2007;133:481-8.

223. Rosselli M, MacNaughtan J, Jalan R, et al. Beyond scoring: a modern interpretation of disease progression in chronic liver disease. Gut. 2013;62:1234-41.

224. Garcia-Tsao G, Friedman S, Iredale J, et al. Now there are many (stages) where before there was one: In search of a pathophysiological classification of cirrhosis. Hepatology. 2010;51:1445-9.

225. Tsochatzis EA, Bosch J, Burroughs AK. New therapeutic paradigm for patients with cirrhosis. Hepatology. 2012;56:1983-92.

226. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet. 2014;383:1749-61.

227. de Franchis R, Baveno VF. Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. J Hepatol. 2010;53:762-8.

228. de Franchis R, Baveno VIF. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. J Hepatol. 2015;63:743-52.

229. D'Amico G, Pasta L, Morabito A, et al. Competing risks and prognostic stages of cirrhosis: a 25-year inception cohort study of 494 patients. Alimentary pharmacology & therapeutics. 2014;39:1180-93.

230. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. JHepatol. 2006;44:217-31.

231. Maurice JB, Brodkin E, Arnold F, et al. Validation of the Baveno VI criteria to identify low risk cirrhotic patients not requiring endoscopic surveillance for varices. J Hepatol. 2016;65:899-905.

232. Augustin S, Pons M, Genesca J. Validating the Baveno VI recommendations for screening varices. J Hepatol. 2017;66:459-60.

233. Augustin S, Pons M, Maurice JB, et al. Expanding the Baveno VI criteria for the screening of varices in patients with compensated advanced chronic liver disease. Hepatology. 2017;66:1980-8.

234. Abraldes JG, Bureau C, Stefanescu H, et al. Noninvasive tools and risk of clinically significant portal hypertension and varices in compensated cirrhosis: The "Anticipate" study. Hepatology. 2016;64:2173-84.

235. Moctezuma-Velazquez C, Saffioti F, Tasayco-Huaman S, et al. Non-invasive Prediction of High-risk Varices in Patients with Primary Biliary Cholangitis and Primary Sclerosing Cholangitis. Am J Gastroenterol. 2018.

236. Sandrin L, Fourquet B, Hasquenoph JM, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol. 2003;29:1705-13.

237. European Association for Study of L, Asociacion Latinoamericana para el Estudio del H. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol. 2015;63:237-64.

238. Arena U, Vizzutti F, Abraldes JG, et al. Reliability of transient elastography for the diagnosis of advanced fibrosis in chronic hepatitis C. Gut. 2008;57:1288-93.

239. Marcellin P, Ziol M, Bedossa P, et al. Non-invasive assessment of liver fibrosis by stiffness measurement in patients with chronic hepatitis B. Liver Int. 2009;29:242-7.

240. Yoneda M, Yoneda M, Mawatari H, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). Dig Liver Dis. 2008;40:371-8.

241. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol. 2008;48:835-47.

242. Tsochatzis EA, Gurusamy KS, Ntaoula S, et al. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. J Hepatol. 2011;54:650-9.

243. Sporea I, Jurchis A, Sirli R, et al. Can transient elastography be a reliable method for assessing liver fibrosis in non alcoholic steatohepatitis (NASH)? Med Ultrason. 2013;15:106-10.

244. Wong VW, Vergniol J, Wong GL, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology. 2010;51:454-62.

245. Roccarina D, Rosselli M, Genesca J, et al. Elastography methods for the non-invasive assessment of portal hypertension. Expert Rev Gastroenterol Hepatol. 2018;12:155-64.

246. Bamber J, Cosgrove D, Dietrich CF, et al. EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 1: Basic principles and technology. Ultraschall Med. 2013;34:169-84.

247. Grgurevic I, Bokun T, Bozin T, et al. Non-invasive diagnosis of portal hypertension in cirrhosis using ultrasound based elastography. Med Ultrason. 2017;19:310-7.

248. Dietrich CF, Bamber J, Berzigotti A, et al. EFSUMB Guidelines and Recommendations on the Clinical Use of Liver Ultrasound Elastography, Update 2017 (Long Version). Ultraschall Med. 2017;38:e16-e47.

249. Friedrich-Rust M, Nierhoff J, Lupsor M, et al. Performance of Acoustic Radiation Force Impulse imaging for the staging of liver fibrosis: a pooled meta-analysis. J Viral Hepat. 2012;19:e212-9.

250. Grgurevic I, Bokun T, Mustapic S, et al. Real-time two-dimensional shear wave ultrasound elastography of the liver is a reliable predictor of clinical outcomes and the presence of esophageal varices in patients with compensated liver cirrhosis. Croat Med J. 2015;56:470-81.

251. Grgurevic I, Puljiz Z, Brnic D, et al. Liver and spleen stiffness and their ratio assessed by real-time two dimensional-shear wave elastography in patients with liver fibrosis and cirrhosis due to chronic viral hepatitis. Eur Radiol. 2015;25:3214-21.

252. Herrmann E, de Ledinghen V, Cassinotto C, et al. Assessment of biopsy-proven liver fibrosis by two-dimensional shear wave elastography: An individual patient data-based meta-analysis. Hepatology. 2018;67:260-72.

253. Koizumi Y, Hirooka M, Abe M, et al. Comparison between real-time tissue elastography and vibration-controlled transient elastography for the assessment of liver fibrosis and disease progression in patients with primary biliary cholangitis. Hepatol Res. 2017;47:1252-9.

254. Godfrey EM, Mannelli L, Griffin N, et al. Magnetic resonance elastography in the diagnosis of hepatic fibrosis. Semin Ultrasound CT MR. 2013;34:81-8.

255. Procopet B, Berzigotti A. Diagnosis of cirrhosis and portal hypertension: imaging, non-invasive markers of fibrosis and liver biopsy. Gastroenterol Rep (Oxf). 2017;5:79-89.

256. Sami SS, Harman D, Ragunath K, et al. Non-invasive tests for the detection of oesophageal varices in compensated cirrhosis: systematic review and meta-analysis. United European Gastroenterol J. 2018;6:806-18.

257. Berzigotti A. Non-invasive evaluation of portal hypertension using ultrasound elastography. J Hepatol. 2017;67:399-411.

258. Ma X, Wang L, Wu H, et al. Spleen Stiffness Is Superior to Liver Stiffness for Predicting Esophageal Varices in Chronic Liver Disease: A Meta-Analysis. PLoS One. 2016;11:e0165786.

259. Colecchia A, Ravaioli F, Marasco G, et al. A combined model based on spleen stiffness measurement and Baveno VI criteria to rule out high-risk varices in advanced chronic liver disease. J Hepatol. 2018;69:308-17.

260. Berzigotti A, Piscaglia F. Ultrasound in portal hypertension--part 1. Ultraschall Med. 2011;32:548-68; quiz 69-71.

261. Samir AE, Dhyani M, Vij A, et al. Shear-wave elastography for the estimation of liver fibrosis in chronic liver disease: determining accuracy and ideal site for measurement. Radiology. 2015;274:888-96.

262. Yoon JH, Lee JM, Joo I, et al. Hepatic fibrosis: prospective comparison of MR elastography and US shear-wave elastography for evaluation. Radiology. 2014;273:772-82.

263. Yoon JH, Lee JM, Woo HS, et al. Staging of hepatic fibrosis: comparison of magnetic resonance elastography and shear wave elastography in the same individuals. Korean journal of radiology. 2013;14:202-12.

264. Lichtinghagen R, Pietsch D, Bantel H, et al. The Enhanced Liver Fibrosis (ELF) score: normal values, influence factors and proposed cut-off values. J Hepatol. 2013;59:236-42.

265. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology. 2003;38:518-26.

266. Giannini E, Risso D, Botta F, et al. Validity and clinical utility of the aspartate aminotransferase-alanine aminotransferase ratio in assessing disease severity and prognosis in patients with hepatitis C virus-related chronic liver disease. Arch Intern Med. 2003;163:218-24.

267. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 2006;43:1317-25.

268. Cross TJ, Rizzi P, Berry PA, et al. King's Score: an accurate marker of cirrhosis in chronic hepatitis C. Eur J Gastroenterol Hepatol. 2009;21:730-8.

269. Ohta T, Sakaguchi K, Fujiwara A, et al. Simple surrogate index of the fibrosis stage in chronic hepatitis C patients using platelet count and serum albumin level. Acta Med Okayama. 2006;60:77-84.

270. Lok AS, Ghany MG, Goodman ZD, et al. Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the HALT-C cohort. Hepatology. 2005;42:282-92.

271. Hsieh YY, Tung SY, Lee IL, et al. FibroQ: an easy and useful noninvasive test for predicting liver fibrosis in patients with chronic viral hepatitis. Chang Gung Med J. 2009;32:614-22.

272. Bonacini M, Hadi G, Govindarajan S, et al. Utility of a discriminant score for diagnosing advanced fibrosis or cirrhosis in patients with chronic hepatitis C virus infection. Am J Gastroenterol. 1997;92:1302-4.

273. Berzigotti A, Seijo S, Arena U, et al. Elastography, spleen size, and platelet count identify portal hypertension in patients with compensated cirrhosis. Gastroenterology. 2013;144:102-11 e1.

274. Bland JM, Altman DG. Measuring agreement in method comparison studies. Stat Methods Med Res. 1999;8:135-60.

275. Ferraioli G, Maiocchi L, Lissandrin R, et al. Accuracy of the ElastPQ Technique for the Assessment of Liver Fibrosis in Patients with Chronic Hepatitis C: a "Real Life" Single Center Study. J Gastrointestin Liver Dis. 2016;25:331-5.

276. Kircheis G, Sagir A, Vogt C, et al. Evaluation of acoustic radiation force impulse imaging for determination of liver stiffness using transient elastography as a reference. World J Gastroenterol. 2012;18:1077-84.

277. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology. 2003;38:1449-57.

278. West J, Card TR. Reduced mortality rates following elective percutaneous liver biopsies. Gastroenterology. 2010;139:1230-7.

279. Filingeri V, Sforza D, Tisone G. Complications and risk factors of a large series of percutaneous liver biopsies in patients with liver transplantation or liver disease. Eur Rev Med Pharmacol Sci. 2015;19:1621-9.

280. Fraquelli M, Baccarin A, Casazza G, et al. Liver stiffness measurement reliability and main determinants of point shear-wave elastography in patients with chronic liver disease. Aliment Pharmacol Ther. 2016;44:356-65.

281. Ferraioli G, Tinelli C, Lissandrin R, et al. Point shear wave elastography method for assessing liver stiffness. World J Gastroenterol. 2014;20:4787-96.

282. Ma JJ, Ding H, Mao F, et al. Assessment of liver fibrosis with elastography point quantification technique in chronic hepatitis B virus patients: a comparison with liver pathological results. J Gastroenterol Hepatol. 2014;29:814-9.

283. Krawczyk M, Ligocka J, Ligocki M, et al. Does transient elastography correlate with liver fibrosis in patients with PSC? Laennec score-based analysis of explanted livers. Scand J Gastroenterol. 2017;52:1407-12.

284. Conti F, Serra C, Vukotic R, et al. Accuracy of elastography point quantification and steatosis influence on assessing liver fibrosis in patients with chronic hepatitis C. Liver Int. 2017;37:187-95.

285. Lammers WJ, van Buuren HR, Hirschfield GM, et al. Levels of alkaline phosphatase and bilirubin are surrogate end points of outcomes of patients with primary biliary cirrhosis: an international follow-up study. Gastroenterology. 2014;147:1338-49 e5; quiz e15.

286. Ponsioen CY, Chapman RW, Chazouilleres O, et al. Surrogate endpoints for clinical trials in primary sclerosing cholangitis: Review and results from an

International PSC Study Group consensus process. Hepatology. 2016;63:1357-67.

287. Bolognesi M, Merkel C, Sacerdoti D, et al. Role of spleen enlargement in cirrhosis with portal hypertension. Dig Liver Dis. 2002;34:144-50.

288. Sharma P, Kirnake V, Tyagi P, et al. Spleen stiffness in patients with cirrhosis in predicting esophageal varices. Am J Gastroenterol. 2013;108:1101-7.

289. Singh S, Eaton JE, Murad MH, et al. Accuracy of spleen stiffness measurement in detection of esophageal varices in patients with chronic liver disease: systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2014;12:935-45 e4.

290. Attia D, Schoenemeier B, Rodt T, et al. Evaluation of Liver and Spleen Stiffness with Acoustic Radiation Force Impulse Quantification Elastography for Diagnosing Clinically Significant Portal Hypertension. Ultraschall Med. 2015;36:603-10.

291. Ali AH, Sinakos E, Silveira MG, et al. Varices in early histological stage primary biliary cirrhosis. J Clin Gastroenterol. 2011;45:e66-71.