This work is dedicated to my wife, my daughter and to all the patients I have met and who helped me through the years in inimaginable ways

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Prospective evaluation of liver and spleen stiffness by transient and point shear wave elastography: surrogate markers of fibrosis and clinically significant portal hypertension in cirrhosis

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SUMMARY

Introduction: Portal hypertension is the hemodynamic consequence of cirrhosis. It is considered a milestone in the progression of chronic liver disease preluding the onset of the most important complications. Elastography is rapidly gaining ground as a non-invasive tool for the diagnosis and characterisation of PH.

Aims: 1. To compare liver stiffness measured by vibration-controlled transient elastography (Fibroscan) with liver stiffness measured by point shear wave elastography (ElastPQ) and evaluate their correlation. 2. To evaluate the diagnostic accuracy of ElastPQ in detecting clinically significant portal hypertension by comparing liver and spleen stiffness with hepatic venous pressure gradient in a population of patients with chronic liver disease. 3. To evaluate the correlation of liver stiffness with liver fibrosis in a subgroup of patients who underwent hepatic venous pressure gradient measurement and liver biopsy. 4. To evaluate the accuracy of liver and spleen stiffness in discriminating cirrhotic portal hypertension from non-cirrhotic portal hypertension.

Patients and Methods: 78 patients with chronic liver disease attending the Royal Free Hospital who underwent hepatic venous pressure gradient measurements for clinical purposes were recruited in the study. Only 70 were enrolled because in 8 there were technical limitations and spleen stiffness could not be measured. The population was heterogeneous in terms of age (59 ± 11), gender (M 78%, F 22%) and aetiology (HCV 17.1%,

HBV 8.6%, NASH 31.4%, ALD 5.7%, other 37.2%). Elastography was measured with ElastPQ (Affiniti 70 G Philips Healthcare) just before the hemodynamic assessment in all 70 participants, and also with vibration-controlled transient elastography (Fibroscan, Echosens, Paris) in a subgroup of 41 patients. Liver stiffness with ElastPQ (13.8), spleen stifness with ElastPQ (40.1), spleen size (13), platelet count (181) and LSPS [liver stiffness*(Spleen diameter/platelet count)] (1.2), were correlated to hepatic venous pressure gradient. Another subgroup of 45 patients had a histopathological sample obtained by transjugular approach at the time of hepatic venous pressure gradient measurement, within 3 months prior to the assessment by percutaneous biopsy or obtained from the resected liver of those patients who underwent surgery for hepatocellular carcinoma. In order to have an objective evaluation of the amount of fibrosis, collagen proportionate area was calculated for every histopathological sample and expressed as percentage related to the area of collagen. Collagen proportionate area was then correlated first with hepatic venous pressure gradient measurement and then with both liver and spleen stiffness measured by ElastPQ. Finally, in order to evaluate the diagnostic accuracy of ElastPQ in characterising portal hypertension, we compared the subgroup of cirrhotic patients affected by clinically significant portal hypertension (26) with a group of patients with non-cirrhotic portal hypertension (21) due to extra-hepatic portal vein obstruction secondary to myeloproliferative neoplasm. Liver stiffness, spleen stiffness, spleen size, spleen stiffness/liver stiffness ratio and platelet count were used as parameters for comparison.

Results: 45 patients underwent both ElastPQ and fibroscan measurements. An excellent correlation between the two techniques was found (Spearman's 0.941, p<0.0001). 26/70 patients (37.2%) had clinically significant portal hypertension (HVPG ≥10 mmHg). Liver stiffness (ElastPQ) (p<0.0001), spleen stiffness (ElastPQ) (p<0.0001), spleen size (p<0.001), platelet count (p<0.0001) and LSPS (p<0.0001) all correlated significantly with clinically significant portal hypertension. However, on multivariate analysis, spleen stiffness was the only parameter independently correlated with clinically significant portal hypertension (OR 1.099, 95% CI 1.017 – 1.188, p<0.017). The spleen stiffness AUROC for HVPG≥10 mmHg was 0.918, p<0.0001, cut off value 42.7 kPa, sensitivity 96%, specificity 84%, negative predictive value 97.4% and positive predictive value 78.1%. Collagen proportionate area was found to have an excellent correlation with hepatic venous pressure gradient (p<0.0001) and was also significantly correlated to liver stiffness measured with ElastPQ (p<0.0001) and spleen stiffness (p<0.005). Finally liver stiffness (p<0.0001), spleen stiffness (p<0.0001), platelet count (p<0.009) spleen size (p<0.001) and spleen stiffness/liver stiffness ratio (p<0.0001) were able to discriminate CPH from NCPH.

Conclusions: In this population of patients ElastPQ was found to have an excellent correlation with Fibroscan which so far has been considered the gold standard of reference for non-invasive measurement of liver fibrosis and portal hypertension. Liver and especially spleen stiffness measured by ElastPQ correlated significantly with hepatic venous pressure gradient being able to discriminate clinically significant portal hypertension from

non-clinically significant portal hypertension with high accuracy. Both liver stiffness and spleen stiffness correlated well with collagen proportionate area which is a true quantitative measurement of liver fibrosis that also correlates faithfully with portal hypertension. Finally it was shown that ElastPQ is particularly useful for distinguishing cirrhotic portal hypertension from non-cirrhotic portal hypertension and overall should be considered as a rapid, accurate and non-invasive method, valuable for assessing liver disease in its multifaceted clinical presentations.

TABLE OF CONTENTS

1. INTRODUCTION

Chronic liver disease is an inflammatory disorder that may progress to fibrosis and cirrhosis if the pathogenic noxae is not withdrawn. The underlying aetiology has surely a role in the pattern of inflammation, distribution of fibrosis and therefore the natural history of liver disease. The histopathological characteristics remain one of the reasons for which liver biopsy cannot and should not be replaced by other methods. Nevertheless non-invasive assessment is based on ultrasound techniques able to describe the liver and spleen appearance as well as the vascular anatomy and flow of the splanchnic circulation. In addition, this "subjective" description can now be integrated by measuring a biomechanical parameter such as tissue stiffness, the use of which is overcoming liver biopsy for staging and follow up purposes. Portal hypertension (PH) represents the hemodynamic consequence of cirrhosis, it is considered a milestone in the progression of liver disease and preludes the onset of the most important complications. Therefore, the management of patients with liver disease relies on prognostic stratification, which is mainly relative to PH. It is within the clinical frame of diagnosis, follow up and staging that the importance of non-invasive assessment is more and more recognised by the hepatology community.

ElastPQ is a fairly new point shear wave elastography (pSWE) technique that has not yet been validated for the assessment of PH. This study aims to establish the correlation between liver stiffness (LS) measured by ElastPQ and vibration-controlled transient elastography (VCTE) in patients who

underwent HVPG measurement for clinical purposes; to evaluate the correlation of ElastPQ in detecting and grading PH by measuring LS and spleen stiffness (SS) in a population of patients with chronic liver disease who underwent haemodynamic assessment; to investigate the correlation between LS measured by ElastPQ and liver fibrosis measured by collagen proportionate area (CPA). Ultimately PH will be classified by comparing LS and SS values measured in a subgroup of patients with cirrhotic CSPH (HVPG ≥ 10 mmHg) and another group of patients with extra-hepatic portal venous obstruction (EHPVO) who were known to have CSPH because of the presence of portal-systemic collateral vessels on cross sectional imaging.

2. DEFINITION AND CLASSIFICATION OF PH

PH is a clinical syndrome characterised by the presence of increased resistance to blood flow in the portal venous system and/or its tributaries (spleno-mesenteric-portal venous axis). According to the site of resistance PH is classified in pre-hepatic, intra-hepatic (pre-sinusoidal, sinusoidal and post-sinusoidal) and post-hepatic (Figure 1). Pre-hepatic portal hypertension is mainly due to EHPVO, usually caused by thrombosis. In the majority of cases liver parenchyma is not affected although there might be signs of arterialisation since a longstanding reduced portal inflow may induce hypertrophy of the hepatic artery and further arterial angiogenesis. A typical feature of EHPVO is the presence of portal vein cavernoma, which is the result of the attempt to re-canalize and bypass the thrombosed portal vein. The spleen is typically enlarged and other portal-systemic vascular collaterals might also be present. Among the intrahepatic causes, cirrhosis

is the most frequent condition, while idiopathic portal hypertension (IPH) and EHPVO account for less than 10%. IPH is typically characterised by splenomegaly, none or mild hepatic fibrosis and the presence of portal-systemic collaterals as a consequence of PH. The liver's heterogeneous echotexture and often abnormal shape may be initially misleading suggesting the presence of cirrhosis which however is excluded by the histological and hemodynamic picture. Intrahepatic causes can be further classified as pre-sinusoidal, sinusoidal or post-sinusoidal according to the site of intrahepatic resistance. Pre-sinusoidal portal hypertension is characterized by increased resistance in the peri-portal areas and can be caused by schistosomiasis, nodular regenerative hyperplasia, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), sarcoidosis. Nevertheless in advanced stages the sinusoidal tracts also may be involved. Post-hepatic portal hypertension is instead associated to venous outflow obstruction which is typically secondary to Budd-Chiari syndrome or to right heart impairment.

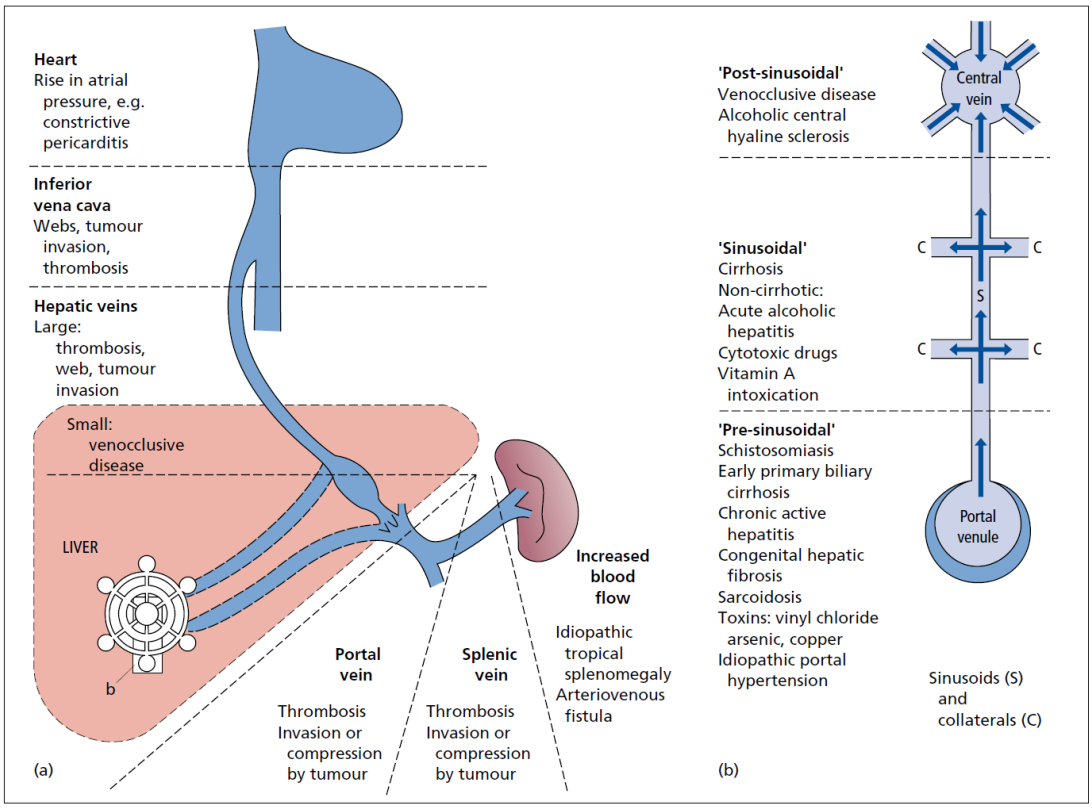

Fig 1. Schematic illustration from the "Sheila Sherlock Book of Hepatology" showing the pathophysiological classification of portal hypertension.

3. **PATHOPHYSIOLOGY OF PH**

PH in cirrhosis develops as a consequence of structural changes of liver parenchyma due to inflammation, collagen deposition, nodule formation and vascular occlusion/remodelling. This "static" component causes the initial vascular modifications responsible for increasing portal pressure. Nevertheless about 1/3 of PH is caused by a functional "dynamic" component [1] which is used by the activation of stellate cells with active contraction of myofibroblasts and vascular smooth muscle cells in portal venules [2]. This in turn is caused by increased endogenous vasoconstrictors, such as endothelin, and reduced nitric oxide bioavailability [3-4]. Porto-systemic collaterals develop as a consequence

of the high pressure in the portal vein and ameliorate the increased resistance. However, even when portal blood flow is entirely diverted through collaterals, PH persists because of a concomitant increase in portal venous inflow, which in turn is caused by splanchnic vasodilatation, [5] mostly mediated by an increase in nitric oxide. The most important collaterals are those that constitute gastroesophageal varices. Although the formation of collaterals had been assumed to be the result of dilatation of pre-existing vascular channels, recent studies have implicated a process of neoangiogenesis. This process has been shown to contribute not only to portal-systemic collaterals but also to the formation of a new arteriolar-capillary network through angiogenesis [6]. While the “static” component increases due to progressive collagen deposition and nodular regeneration, the “dynamic” component increases progressively intrahepatic resistances, splanchnic vasodilation and inflow that is diverted through portal-systemic collaterals. This results in further recruitment of vascular shunts, splenomegaly and further systemic increase of vasodilators which ultimately leads to a mismatch of vascular resistances and redistribution of blood volume. The neuro-hormonal modifications, triggered mainly by renal hypoperfusion, maintain the vicious cycle and actively contribute to the pathogenesis of hyperdynamic circulation that is the consequence and cause of further establishment of haemodynamic abnormalities that characterize cirrhosis in its most advanced stages.

4. NATURAL HISTORY

The relevance of PH derives from the frequency and severity of its complications, which represent the first cause of hospitalisation, liver transplantation and death. These complications include the formation of oesophageal or gastric varices, variceal bleeding, ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, portopulmonary hypertension, hepatic encephalopathy, portal hypertensive gastropathy, enteropathy and altered metabolism of endo and xenobiotics normally metabolised by the liver [7].

All patients with cirrhosis will eventually develop PH and gastroesophageal varices. Bleeding from ruptured varices is the most threatening complication of cirrhosis and is the cause of death in about one third of patients. The rate of development and growth of oesophageal varices is poorly defined but in general seems to be related to the degree of liver dysfunction. Once varices have formed, they tend to increase in size and eventually to bleed. Variceal size is the single most important predictor of a first variceal bleeding episode. The risk of hemorrhage is greatest in the first days following a bleeding episode and slowly declines thereafter. Varices can also be found in the stomach of cirrhotic patients, alone or in association with esophageal varices. Gastric varices bleed less frequently but more severely than esophageal varices. Portal hypertensive gastropathy is a common feature of cirrhosis, and its prevalence parallels the severity of PH and liver dysfunction. Portal hypertensive gastropathy can progress from mild to severe and vice-versa or even disappear

completely. Acute bleeding from portal hypertensive gastropathy seems to be relatively uncommon, and less severe than bleeding from varices [8].

The differences between CPH and NCPH are not only topographical and pathophysiological; there is also a huge difference in terms of natural history and prognosis. While NCPH includes a variety of different pathologies and is often an occasional finding in the context of an underlying condition, PH in cirrhosis has a clear role and is used for prognostic stratification. Moreover while in NCPH the liver might be healthy, in cirrhosis it is obviously severely affected and a bleeding episode has an increased risk of hepatic decompensation and death.

5. DIAGNOSIS OF PH

5.1 CLINICAL PRESENTATION

Chronic liver disease usually progresses in an indolent manner until complications, in general secondary to CSPH, do not occur. Ascites, pitting edema, palmar erythema, spider naevi, gynecomastia, abdominal wall collateral circulation, splenomegaly are all signs of advanced cirrhosis. However chronic liver disease typically does not declare itself when portal hypertension is within the subclinical range of 6-9 mmHg and sometimes even if the HVPG threshold of CSPH is reached (>10 mmHg). Nevertheless, after this stage is reached, signs and symptoms may present gradually or as a dramatic event such as variceal bleeding. Hence while a positive clinical

examination is highly specific, a negative one may hide the presence of cirrhosis. In conclusion in a patient with a medical history of chronic liver disease, a negative physical examination has poor diagnostic accuracy and cannot be trusted. Its usefulness is instead relevant when CSPH leads to known stigmata of cirrhosis (ascites, palmar erythema, splenomegaly, spider naevi).

5.2 **SEROLOGY**

Several serum markers have shown to be able to reflect the underlying changes of hepatic dysfunction, fibrosis, cirrhosis and PH. Some of these markers are non-specific tests (indirect or surrogate markers), which combined together increase their accuracy in predicting fibrosis and related PH. Among these surrogate markers the AST to platelet ratio score (APRI) and the Fibrotest are used in clinical practice. The FibroTest, which is a combination of α2-macroglobulin, ApoA1, Bilirubin, γGT, haptoglobin measurements, is the most validated indirect test for liver fibrosis [9-11]. Nevertheless, although these markers have shown a good correlation with advanced stages of liver disease they have not been proved useful in distinguishing different stages of fibrosis, resulting inadequate tests for monitoring the progression of liver disease. As for PH, Child-Pugh score and its objective component (albumin, bilirubin, INR) correlate with HVPG [11-14] and with the prevalence and grade of esophageal varices in cirrhotic patients. Interestingly this correlation is observed also in patients with compensated cirrhosis [15], suggesting that a close relationship exists between the structural changes that give onset to PH and hepatocellular

dysfunction. Platelet count is independently correlated with the prevalence and grade of esophageal varices in several studies [16-17] and platelet count to spleen diameter ratio >909 has been shown to have a 100% negative predictive value for the presence of esophageal varices [18] suggesting that it could be of help in avoiding unnecessary endoscopies. A second panel is represented by direct markers that reflect the constituents of the extracellular matrix released in the blood stream as a consequence of the remodeling process (fibrogenesis and fibrinolysis) [19]. The enhanced liver fibrosis (ELF) score is a combination of hyaluronic acid, tissue inhibitor of matrix metalloproteinases-1 and aminoterminal propeptide of type III procollagen, all of which have shown good accuracy in being able to distinguish different stages of fibrosis and in particular advanced fibrosis and cirrhosis [20]. Lately a recent study has shown how the results may vary also according to sex, age, BMI and therefore these differences should be considered when interpreting ELF test [21]. Guechot and colleagues looked at the predictive value of hyaluronic acid in a series of patients with HCV cirrhosis, followed up for a median of 38 months [22]. In this study, hyaluronic acid had a predictive value equivalent to Child-Pugh score for the prediction of severe complications of cirrhosis or death and therefore for the severity of PH. Several serological markers have been proposed for the detection of CSPH including [23] a score combining total bilirubin and platelet count and Fibrotest [23-25]. Similarly other biological markers were used to study the correlation with the presence of oesophageal varices. Of these the Lok and Forns index showed the best predictive value [26].

5.3 **ENDOSCOPY**

Variceal bleeding is the most life-threatening complication of cirrhosis. Hence once the diagnosis of cirrhosis is made, gastroscopy is the primary investigation because of its sensitivity, specificity and potential therapeutic approach. There are two moments in the follow-up of patients with chronic liver disease in which endoscopy is absolutely crucial: at the time of diagnosis of cirrhosis and once varices are detected, since the grade and risk of bleeding (defined by the size and presence of wale marks and red spots) influence the kind of endoscopic approach which will be eradication or follow-up. Nevertheless, endoscopy is an expensive and uncomfortable procedure and knowing when is the best time to start screening a specific patient is very important. Non-invasive assessment by VCTE has provided useful insights and has recently been considered the standard of reference in adjunction to platelet count to decide when cirrhosis is complicated by CSPH and is therefore likely associated to the development of gastroesophageal varices (Baveno VI Consensus Conference), giving indication when patients should undergo variceal screening and when they could safely avoid it. However, this evaluation was carried out mainly in patients with viral-related cirrhosis and this is not representative of the whole spectrum of liver disease. Moreover it is important to highlight that endoscopy does not diagnose PH but it describes its expression in the most frequent and dangerous anatomical site of vascular collateral circulation development. In a minority of cases patients will develop ectopic varices which could be located at the level of the duodenum or even in the small

bowel with an equally threatening significant risk of bleeding. Alternatively, in the presence of cirrhosis with suspected CSPH and a negative endoscopic evaluation, the presence of vascular shunting with decongestion of the portal venous system should always be borne in mind.

5.4 **HAEMODYNAMIC ASSESSMENT**

Haemodynamic assessment of PH sees its origins more than 60 years ago. Initially portal pressure was measured by direct puncture of the portal vein. However this was invasive and inconvenient and carried a significant risk of complications. In 1951, Myers and Taylor first described wedge hepatic venous pressure (WHVP), which is the measurement of the sinusoidal pressure obtained by occlusive hepatic vein catheterisation, an indirect measurement of portal venous pressure (PVP) [27]. In 1954 Atkinson and Sherlock carried out a pioneering study to measure and characterise PH in patients with cirrhosis and EHPVO [Fig 2] [28]. The rationale was based on the fact that the splenic red pulp is in direct communication with the splenic portal free radicals which drain in the portal venous system, hence the presence of PH would be transmitted and therefore measurable through the spleen. “Patients were placed recumbent with their left arm behind their head. A site was chosen in either the 8th or 9th intercostal space in the mid-axillary line. After injecting local anaesthetic a fine 7 cm lumbar puncture needle was introduced 2 cm into the spleen and the needle was connected to a pressure system. Contemporarily, a WHVP was carried out to evaluate the difference of

hepatic contribution". While intrasplenic pressure was raised in both cohorts of patients with PH with no signficant difference between the two groups, the WHVP instead showed a considerable difference being able to clearly distinguish patients with CPH from those with NCPH. In 1957 a similar but more extensive study was carried out by Turner et al to further corroborate the previous results and investigate the clinical applications of these techniques [29]. The study was carried out on a population of 109 subjects composed by patients with cirrhosis, NCPH and splenomegaly of unknown cause. Percutaneous intrasplenic pressure, trans-splenic venography of the portal venous system and WHVP were measured for each participant. The authors first showed that intrasplenic pressure does not change if measured in different sites, meaning that portal pressure is homogeneously transmitted to the red pulp [Fig 3].

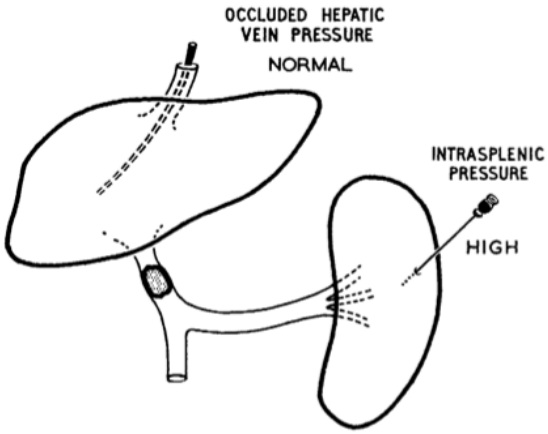

Fig. 2. The drawing illustrates the technique used for indwelling pressure measurements of both liver and spleen in a patient with portal hypertension due to extra-hepatic portal vein obstruction [28].

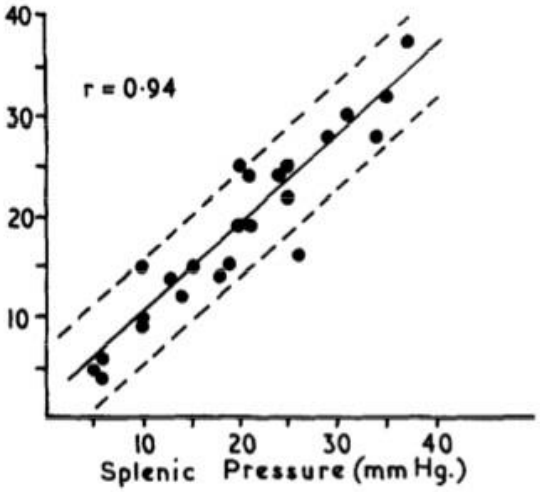

Fig. 3 . The relationship between pressure recorded at two different sites in the same spleen [29].

The results showed also that, in general, vascular collaterals were associated to increased intrasplenic pressure as an expression of PH, and that intrasplenic pressure was high in the majority of patients who had a variceal bleed compared to those who did not. However, it was also shown that some patients with no collateral circulation had high intrasplenic pressure, while in some with very large vessles it was low [Fig 4]. "In one patient with cirrhosis the intrasplenic pressure fell spontanously from 25 mmHg to 12 mmHg in twelve months. A venogram at the time of the second pressure measurement showed enormous oesophageal vessels which had presumably lowered portal hypertension". This result suggests that the splenic pressure is relieved by the presence of a natural or iatrogenic collateral vascular circulation as proven by measuring intrasplenic pressure in patients before and after undergoing a porto-caval shunt. In the latter scenario intrasplenic pressures were significantly lower after the procedure, while they remained high in the case of blockage or shunt

mulfunction [Fig 5]. Therefore splenic pressures could be used as a predictor of treatment response. In order to examine the relationship of various hemodynamic parameters, years later Sarin et al measured the correlation between WHVP, intrahepatic interstitial pressure, intrasplenic pressure and intravariceal pressure in patients with CPH and NCPH showing an excellent correlation between intrasplenic and intravariceal pressure in both populations. Hepatic pressures were instead significantly higher in the cirrhotic population compared to the other, as expected [Fig 6-8].

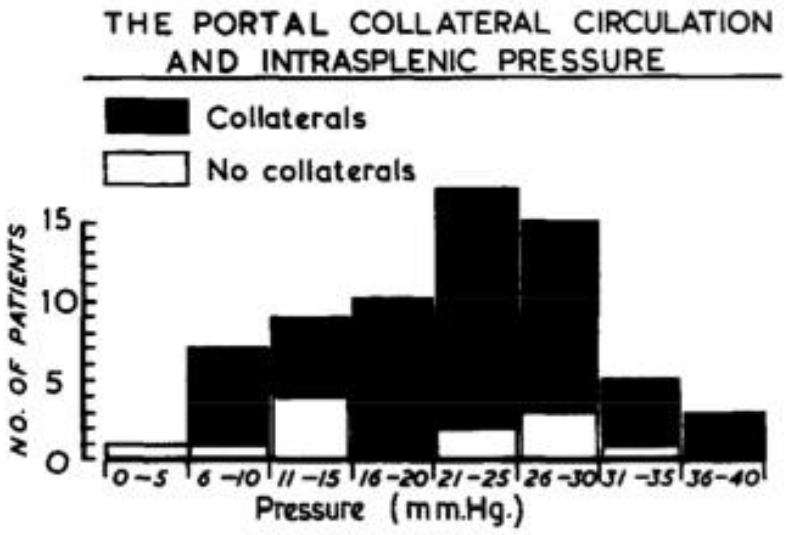

Fig 4. The correlation between the intrasplenic pressure and the presence or absence of vascular collaterals in cirrhotic patients [29].

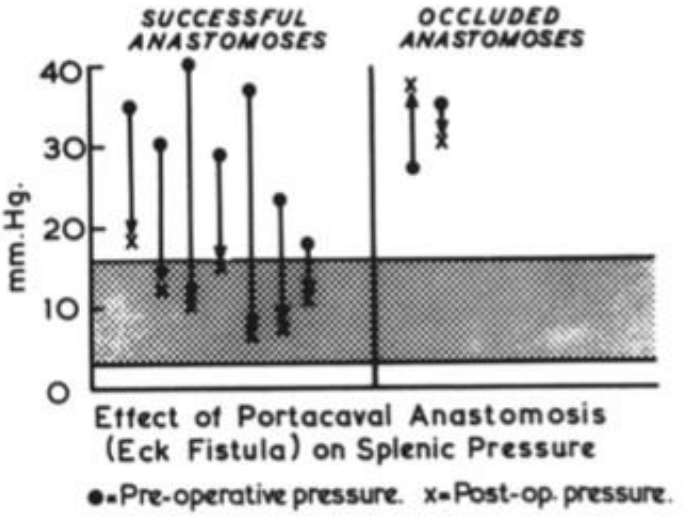

Fig 5 . Succesful porto-caval anastomosis is followed by a fall in intrasplenic pressure. Hatched area represents normal range of intrasplenic pressure. Readings were compared with venography result [29].

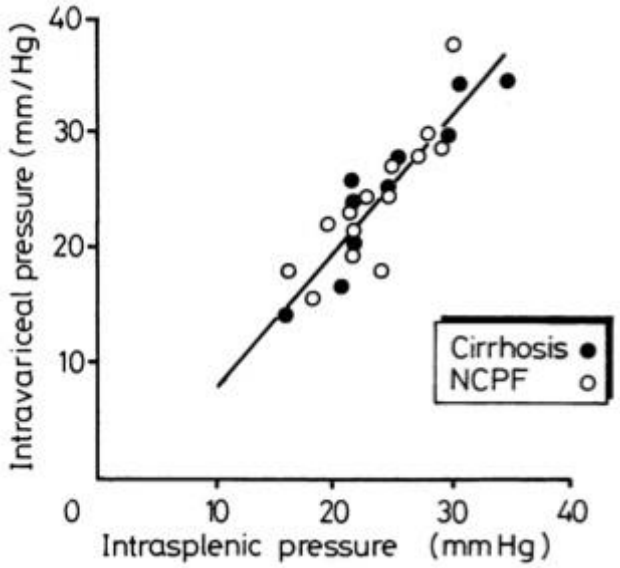

Fig 6. Intrasplenic and intravariceal pressure in patients with CPH and NCPH [29].

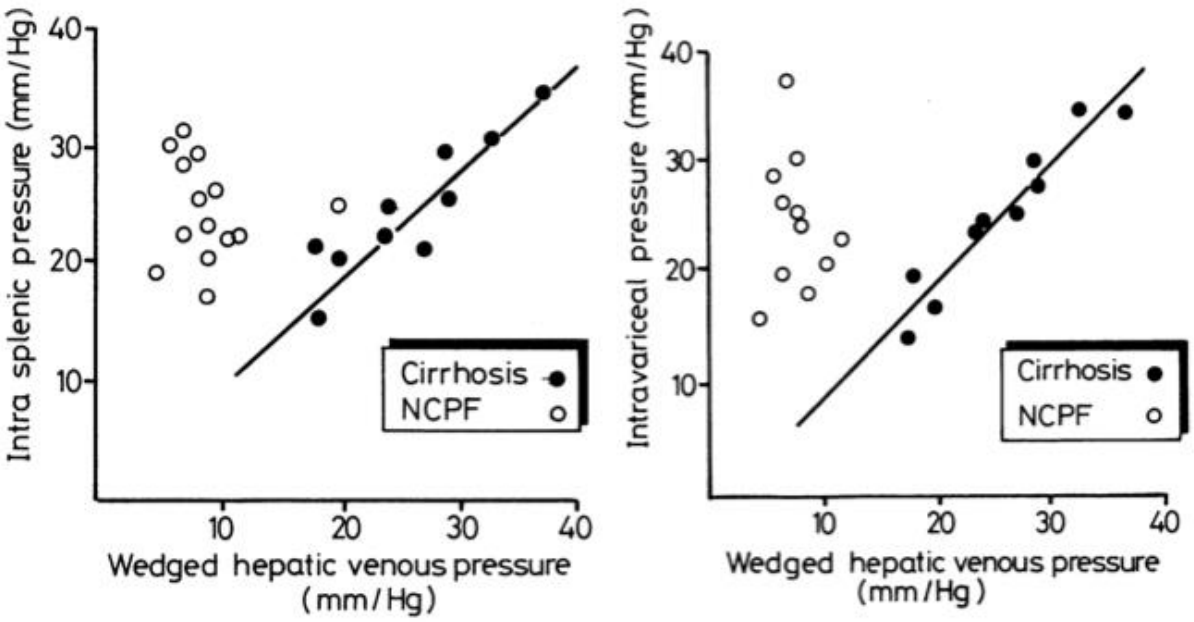

Fig 7. Wedged hepatic pressure and intrasplenic pressure in CPH and NCPH (left). Wedge hepatic pressure and intravariceal pressure in CPH and in NCPH (right) [29].

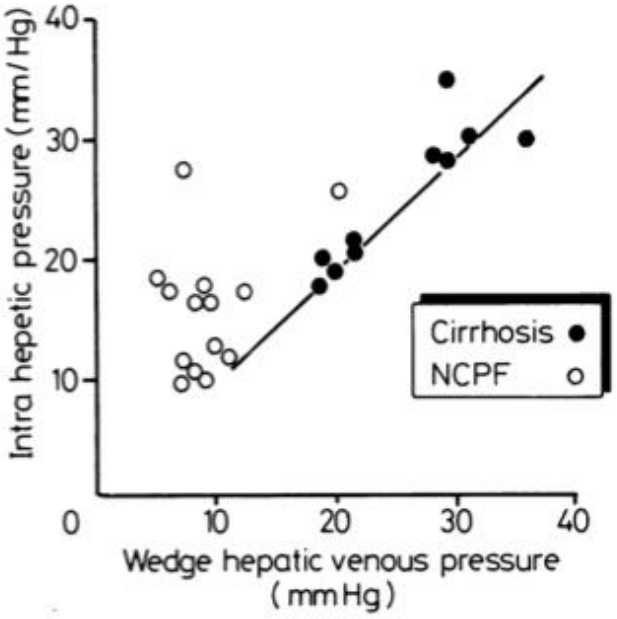

Fig 8. Wedge hepatic pressure and intrahepatic pressure in CPH and NCPH [29].

Several subsequent studies showed similar correlations and served as further proof and validation of these methods. Nevertheless, splenic puncture and intravariceal puncture are particularly invasive and carry a

high risk of complications. However, the possibility of measuring variceal pressure was not abandoned, and in 1996 Nevens et al [30] measured variceal pressure with a specific device built in the endoscope. A subgroup of patients scheduled for variceal sclerotherapy also underwent intravariceal pressure measurement. A good correlation was found between the invasive and the non-invasive pressure measurements with the grade of varices and risk of bleeding expressed as red colour signs. While intrasplenic pressure measurement has been progressively abandoned, variceal pressure is still being investigated and increasingly refined technologies are being developed to non-invasively assess pressure within the varices and to monitor pharmachological response [31]. Although variceal pressure measurement is promising and was proposed recently by some authors as an alternative to other hemodynamic measurements, the gold standard for the measurement of PH remains HVPG, which is defined as the difference between the WHVP and the free hepatic venous pressure (FHVP). It is based on the concept that when the blood flow in a hepatic vein is blocked by a 'wedged' catheter, the static column of blood transmits the pressure from the preceding communicated vascular territory. In the normal liver, interconnected sinusoidal network partially dissipates the pressure backup from the wedged catheter, and the WHVP is slightly lower than directly-measured portal pressure. In cirrhosis the intersinusoidal communications are lost due to fibrosis, septa and nodule formation and the sinusoidal pressure equilibrates with portal pressure reliably [Fig 9]. Moreover, HVPG represents the gradient between the portal vein and the intra-abdominal vena caval pressure. In fact, portal

venous pressure or WHVP can be elevated falsely in the presence of ascites and elevated intra-abdominal pressure. Instead, since both the WHVP and FHVP are affected equally by intra-abdominal pressure, their gradient is not, reflecting faithfully possible increases in portal pressure.

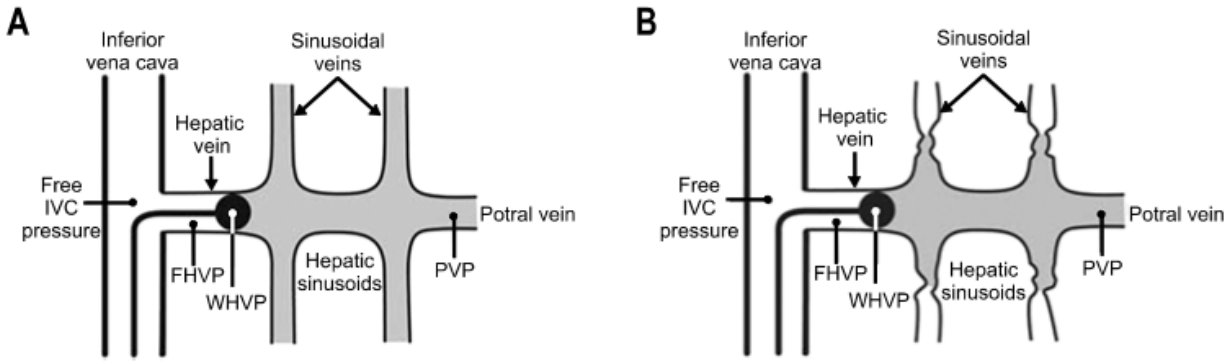

Fig 9. HVPG measurement in normal liver (A) and cirrhotic liver (B) [32].

5.4.1 **Clinical Applications of HVPG**

HVPG is the gold standard for the measurement but also for the classification of PH. In patients with PH of unknown causes the finding of an increased HVPG owing to an increase in WHVP indicates an increase in sinusoidal pressure, which is most frequently due to cirrhosis. A normal HVPG with normal WHVP and FHVP is typical of presinusoidal portal hypertension such as schistosomiasis, early PBC, nodular regenerative hyperplasia [33]. Because the catheter in these cases is not in continuity with the area of increased resistance, the recorded pressure will be that of the normal sinusoids and not of the increased pressure in the portal vein underestimating portal pressure. In IPH the precise location of resistance

to portal venous flow is not exactly known. Two specific pathological lesions observed in this disease include occlusive changes in the intrahepatic portal vein radicles which are focal in distribution and diffuse collagenisation of the space of Disse. Unless the fibrotic process does not extend to the sinusoids, PH is typically periportal and presinusoidal. Hence the wedge pressure is low in these patients [34-35]. In post-hepatic portal hypertension HVPG will be normal because both FHVP and WHVP will be increased. In addition HVPG measurement use has shown to go beyond diagnosis and classification. It has been extensively studied in the course of the natural history of cirrhosis, it is now recognised as the best surrogate marker of clinical events and is considered the best method for prognostic stratification of patients with CLD [36].

5.4.2 **HVPG for staging chronic liver disease**

Once the cirrhotic stage is reached, chronic liver disease can be subclassified according to the presence or absence of vascular collateral circulation and clinical decompensation defined by the development of ascites, variceal hemorrhage, encephalopathy, and jaundice. In general portal pressure is normal when HVPG is between 1 and 5 mmHg, not clinically significant between 6 and 9 mmHg, while the threshold of CSPH is instead defined by an HVPG ≥ 10 mmHg. Although this is generally considered the limit beyond which vascular collateral circulation develops, patients with an HVPG of 10 mmHg are rarely clinically symptomatic, are compensated and the mortality risk is low. Nevertheless it is also reported

that these patients do have a 22% risk of clinical decompensation at 2 years [37] suggesting that prognosis starts to be heavely influenced once this threshold is reached. An HVPG ≥12 mmHg instead is classically associated with an increased risk of bleeding. Whether an increased mortality risk is observed when HVPG is above 16 mmHg [38-39] and during acute variceal bleeding a HVPG >20 mmHg (measured within 48 h of admission) predicts failure to control bleeding and low 1-year survival [40]. In patients with decompensated cirrhosis listed for liver transplantation HVPG holds prognostic value independent from that of model for end-stage liver disease (MELD) score [41]. Pre-operative portal pressure is an important predictor of hepatic decompensation in patients with cirrhosis after resection for HCC. Bruix et al evaluated that only HVPG was significantly associated with unresolved decompensation within 3 months after surgery [42-43]. Therefore, preoperative HVPG should be measured routinely in these patients [33]. Nevertheless, although HVPG is considered the gold standard for measuring portal pressure, it must be highlighted that the onset of CSPH and its related complications might be different according to the underlying aetiology and related pathopysiology as well as topographic distribution of fibrosis. Therefore, it should always be borne in mind that aetiologies which are associated to initial peri-portal fibrosis such as PBC or PSC may have an early onset of PH, that this is pre-sinusoidal and hence it is associated to an underestimation of PH mesured by the HVPG.

5.5 **IMAGING**

5.5.1 **CT and MRI**

Computed tomographic scan (CT) and magnetic resonance (MRI) allow an accurate visualization of the liver parenchyma and the portal venous system. However, while single or multi-detector CT are reliable in detecting large esophageal varices (specificity 90–100% and sensitivity 84–100%), the sensitivity for small varices detection is lower [44]. Dynamic contrast-enhanced single-section CT scans and MRI and phase contrast MR angiography allow a quantitative measurement of portal [45] and azygos [46] blood flow. Azygos blood flow correlates with the presence of esophageal varices at endoscopy, and with the risk of bleeding from varices. Portal fraction of liver perfusion and mean transit time at MRI, have been recently shown to have a good correlation with HVPG [47]. MRI has the advantage over CT of offering high contrast resolution without exposure to ionizing radiation or to large volumes of iodinated contrast media. In addition MRI elastography by synchronizing motion-sensitive imaging sequences with the application of acoustic waves in tissue media, is able to measure tissue response to an applied physical stress [48]. MRI elastography has been shown to be able to predict the stage of liver fibrosis in patients with chronic liver disease [49] and has been successfully applied to measure SS, which seems to have a closer correlation with portal pressure [50]. However, although MRI is a truly multi-parametric and excellent method to evaluate liver disease giving both qualitative and

quantitative information, it is very expensive, not available to every Centre and time consuming.

5.5.2 **Ultrasound**

Ultrasound is the first line examination used to assess and follow up liver disease including hepatocellular carcinoma screening. It is repeatable, not expensive and can be performed at the patient's bedside. Moreover, with the latest technical advancements it truly has become a multi-parametric diagnostic tool. It can provide morphological information on liver appearance, on splanchnic blood flow (direction, velocities and impedance indexes), on tissue stiffness and, by using contrast enhanced software, it can provide fundamental information on the characterization of focal liver lesions as well as on parenchymal microperfusion. Therefore in expert hands it can surely give precious diagnostic and prognostic information on liver disease.

The accuracy of ultrasound diagnostic performance is based on the combination of different sonographic signs [Table 1]. A basic gray scale analysis is important for the description of the parenchymal appearance. Size, shape, echo-texture and outline are the first findings to be described when assessing liver disease. Liver surface nodularity, although not exclusive, is one of the most specific signs of cirrhosis [51]. Nevertheless the sensitivity of single ultrasound findings is low. Interrogation of the liver vascular anatomy and spleen is extremely important and provides further information in order to increase ultrasound diagnostic accuracy.

Ultrasound signs of CSPH might be very specific, but their sensitivity is low especially in compensated cirrhosis; therefore, while the presence of a sign or a combination of signs definitely rules-in PH, its absence cannot exclude the diagnosis within certain limits (Table 1). When intrahepatic resistance is greater than the resistance of portal-systemic collaterals there is an inversion of portal blood which is 100% specific for PH, as well as the presence of portal-collateral circulation such as para-umbilical vein recanalization, spontaneous spleno-renal circulation, dilated left and short gastric veins [52]. Other ultrasound signs of CSPH include dilatation of portal vein (diameter >13 mm) [53]. Some authors have reported that a portal vein dilatation above 12 mm has a specificity of 95% for the diagnosis of PH in chronic liver disease, and has been consistently associated with esophageal varices. However in some cases portal venous caliber even in the presence of PH is normal. These differences may be related to the underlying cause of liver disease. Portal vein blood velocity can be assessed with good reproducibility. It usually decreases as portal pressure increases in cirrhosis as a consequence of the increased resistance to inflow. A maximum velocity below 16 cm/s and a mean below 12-10 cm/sec should be considered strongly suggestive of CSPH [54]. The congestion index combines PV velocity and PV cross sectional area and has been related with the presence of esophageal varices [55]. Altered hepatic venous Doppler pattern [56], increased intra-parenchymal hepatic and splenic artery impedance [57–59], increased intra-parenchymal renal artery impedance [60] and reduced mesenteric artery impedance [61] are influenced by the presence of hyperdynamic circulation which is a

consequence of advanced cirrhosis. HVPG significantly correlates with some ultrasound parameters such as portal vein velocity and volume of blood flow [54], hepatic artery resistance index, splenic and renal artery resistance and pulsatility index. However the degree of correlation is only slight to moderate and these parameters cannot be used as reliable surrogates of HVPG [53]. Dilatation of splenic and mesenteric vein, and the reduction of the respiratory variations of their diameter are instead very specific signs of CSPH [61]. Ultrasound is highly sensitive in diagnosing ascites, which is the most common clinical decompensation event of cirrhosis and holds a severe prognostic significance [53]. Splenomegaly often accompanies the development of PH [62] in cirrhosis and is considered a physical stigmata of advanced chronic liver disease. In general it is thought to be associated with a more severe disease since it is more often observed in decompensated than compensated patients [63] as well as in patients with esophageal varices. As for the prediction of first clinical decompensation of any kind, spleen enlargement (>1 cm) on follow-up might be associated with a higher probability of developing the first clinical decompensation of cirrhosis [64]. However, spleen size sometimes may not correlate with the severity of PH even in case of advanced liver disease. This finding seems to be aetiology-related [65]. Ultrasound with color Doppler analysis is also particularly useful as a diagnostic guide for non-cirrhotic causes of PH. In patients with no history of chronic liver disease but clinical signs of PH, particular attention should be payed to vessel patency. The presence of portal vein thrombosis and cavernomatous transformation is a pathognomonic sign of NCPH secondary to EHPVO. In

patients with no ultrasound signs of cirrhosis and patent portal and hepatic veins, the observation of signs of PH should suggest rare causes of PH such as arterial-portal fistulae, IPH or nodular regenerative hyperplasia [66]. Color Doppler ultrasound also permits an evaluation of the hepatic veins and the inferior cava vein, thus allowing the identification of possible post-hepatic causes of PH, such as hepatic vein thrombosis (Budd-Chiari syndrome) in which caudate lobe hypertrophy can be very pronounced, with consequent compression of the retro-hepatic vena cava [67]. Right heart failure, tricuspid valve diseases and constrictive pericarditis can also induce PH and ultrasound Doppler is useful in outlining signs of increased central venous pressure such as dilatation of the inferior vena cava and the hepatic veins as well as the distortion of the spectral waveform. Ultrasound is also useful in the assessment of more complex and rarer causes of PH, such as sinusoidal obstruction syndrome and in patients with PH due to suspected hereditary hemorrhagic telangiectasia, in which an increased diameter of the common hepatic artery (>7mm), increased hepatic artery flow and the presence of intrahepatic hypervascularization and subcapsular vascular spots with a high-velocity arterial blood flow and low resistivity index, are highly sensitive and specific [68–70].

		sensitivity	specificity
portal venous system	dilatation of portal vein (≥ 13 mm)	< 50%	90 – 100%
	reduction of portal vein blood flow velocity (time averaged mean vel. < 14 – 16 cm/sec[2])	80 – 88%	80 – 96%
	reversal of portal vein blood flow	not reported; sign prevalence: 8.3% of unselected pts	100%
	increased portal vein congestion index (≥ 0.08)	67 – 95%	100%
	dilatation of splenic vein (SV) and superior mesenteric vein (SMV) (≥ 11 mm)	72%	100%
	reduction of respiratory variation of diameter in SV or SMV (< 40%)	79.7%	100%
spleen	splenomegaly (diameter > 12 cm and/or area ≥ 45 cm[2])	93%	36%
splenic artery	increased Doppler resistive index or impedance index of the intraparenchymal branches (RI ≥ 0.63, PI ≥ 1.00)	84.6%	70.4%
hepatic artery	increased Doppler resistive index of the intrahepatic branches (> 0.78)	50%	100%
renal artery	increased Doppler resistive index of the right interlobar renal artery (≥ 0.65)[4]	79.5%	59.3%
SMA	decreased Doppler pulsatility index (≤ 2.70)	85.7%	65.2%
presence of porto-systemic collateral circulation		83%	100%

Table 1. Main reported US and Doppler ultrasound signs of portal hypertension in patients with chronic liver diseases [66]

5.6 **ELASTOGRAPHY**

5.6.1 **VCTE**

In general, liver biopsy provides only a very small part of the whole organ and there is a risk that this part might not be representative for the amount of liver fibrosis affecting the liver due to heterogeneity in its distribution [71]. Besides technical problems, liver biopsy remains a costly and invasive procedure that requires physicians and pathologists to be sufficiently trained in order to obtain adequate and representative results. Moreover, liver biopsy is an invasive procedure, carrying a risk of rare but potentially life-threatening complications [72,73]. These limitations have led to the development of non-invasive methods for the assessment of liver fibrosis. VCTE has been the first method to be employed and has now taken over

liver biopsy for the staging and follow up of patients, especially with HCV-related chronic liver disease. VCTE measurements are performed with an ultrasound transducer probe built on the axis of a vibrator by which a vibration of mild amplitude and low frequency is transmitted, inducing a wave that propagates through the liver tissue (Fig 11). Pulse-echo acquisitions are performed to measure the velocity of propagation of the wave, which is directly related to tissue stiffness. The volume of the analyzed liver is about 100 times greater than that obtained by biopsy, and has therefore a potentially lower sampling error. Since fibrosis within the liver increases the organ's stiffness, VCTE has been used to assess the presence of fibrosis and cirrhosis and most lately for predicting the presence of CSPH. Nevertheless there is conflictual data on the cutoff values that indicate the presence of cirrhosis and CSPH. The reasons of these discrepancies are likely to be aetiology-driven and related to the pathophysiology of PH. The distribution of fibrosis is in fact dependent on the underlying cause and the characteristics of the inflammatory process. Nodular regeneration ranges from micro to macronodular and this has an impact on the distribution and amount of fibrosis between the nodules and vascular architectural distortion. Since fibrous tissue is the main responsible factor of liver stiffness, the amount but also the topographic distribution of fibrosis will influence these values. Hence different liver disease aetiologies will have different cutoff values for grading liver disease. Although fibrosis is the main drive of increased stiffness, the liver is an organ with a distensible but non-elastic envelope (Glisson's capsule) and additional space-occupying tissue abnormalities, such as edema,

inflammation, extra-hepatic cholestasis, or congestion, can interfere with measurements of LS, independently from fibrosis, by increasing intra-hepatic pressure. Therefore the results should always be interpreted bearing in mind these potential confounding elements [74]. Several studies have shown that there is a good correlation between LS values and HVPG in patients with advanced liver diseases in both pre- and post-transplant settings [75-77]. According to available data, the diagnostic performance of VCTE for predicting CSPH (HVPG >10 mmHg) in the setting of patients with compensated chronic liver disease/cirrhosis is excellent, with an AUROC of 0.93 [78]; a 90% sensitivity cut-off for CSPH diagnosis is 13.6 kPa, and a 90% specific cut-off in this setting is 21 kPa. These cut-offs have been shown to allow a correct stratification of presence/absence of CSPH in patients with compensated cirrhosis and potentially resectable HCC, thus reducing the need for invasive hemodynamic assessment [79]. However, while the correlation is excellent for HVPG values between 5 and 10– 12 mmHg, it hardly reaches statistical significance for values above 12 mmHg [Fig 10] [80-81].

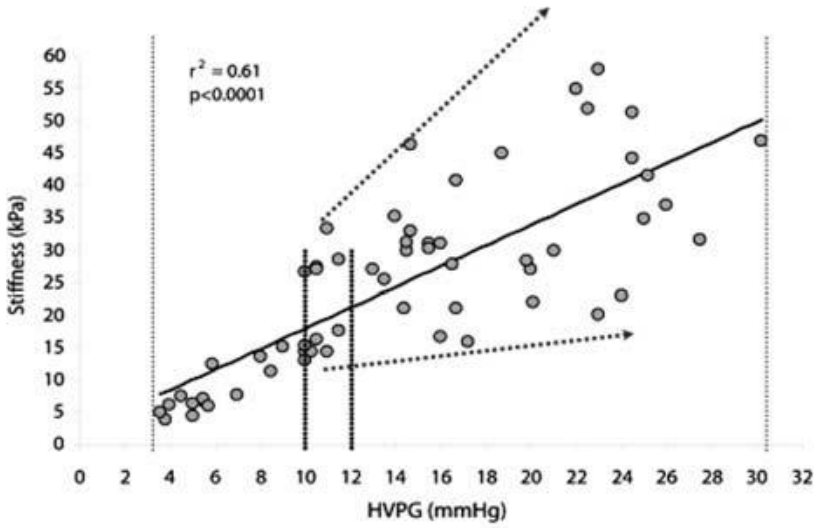

Fig 10. The correlation between liver stiffness and HVPG. Above 10-12 mmHg the correlation is lost [81].

This is because, with the progression of cirrhosis, the mechanisms of PH become less dependant on the intra-hepatic resistance to portal flow due to tissue fibrosis, and progressively more dependant on extra-hepatic factors [82], and since the presence, size and associated risk of bleeding depend on high HVPG measurements, LS is not a reliable parameter to monitor or predict these endpoints. This observation sets a key limitation to the use of LS measurements as a non-invasive surrogate of HVPG beyond the prediction of clinically significant (HVPG 10 mmHg) and severe PH (HVPG ≥12 mmHg). Recently, studies employing different technical approaches have highlighted the potential usefulness of SS assessment for predicting the presence of esophageal varices and the degree of PH in cirrhotic patients [83-86].

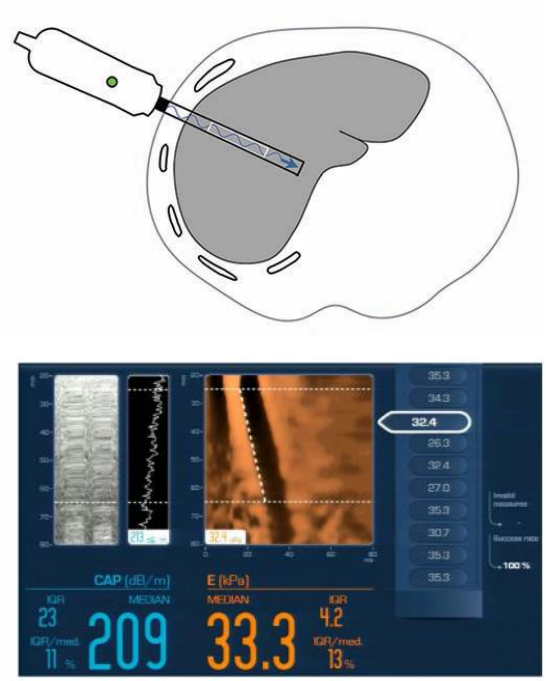

Fig 11. VTCE: a pulsed vibration is delivered longitudinally through the subcutaneous tissue and into the liver for a length of 4 cm X 1 cm. The monitor on the left displays the M-Mode, artifacts and the elastogram together with the mean stiffness value and the interquartile range (IQR) and IQR/Med. The controlled attenuation parameter (CAP) is also shown on the left bottom side of the monitor and is related to the hepatic fat content.

5.6.2 **Point Shear Wave Elastography**

The accuracy of ultrasound imaging in diagnosing liver disease has increased enormously by giving the possibility to obtain biomechanical measurements that reflect the underlying pathophysiological process. LS and most lately SS can be measured, providing precious information within the same baseline ultrasound assessment. Non-invasive evaluation of liver fibrosis has been increasingly used over the last years. Recently, the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) issued guidelines regarding the clinical application of these techniques [87,88]. According to these guidelines, ultrasound-based elastographic techniques are classified in: strain techniques and shear wave elastography techniques. Three types of elastographic techniques are

included in the last category: VCTE (as described above), Point Shear Wave Elastography (pSWE) and shear wave elastography (SWE) imaging (including 2D-SWE and 3D-SWE). In the pSWE category two techniques are included: Acoustic Radiation Force Impulse (ARFI) and ElastPQ (Point Quantification). With ARFI, the ultrasound probe produces an acoustic "push" pulse that generates shear-waves that propagate into the tissue. Their speed, measured in meters/second, is displayed on the screen and reflects the underlying tissue stiffness, the propagation speed increasing with tissue stiffness. Using image-based localization, shear wave speed may be quantified in a precise anatomical area, focused on a region of interest, with a predefined size, provided by the system [89,90]. ElastPQ system generates an electronic voltage pulse, which is transmitted to the transducer. In the transducer, a piezoelectric array converts the electronic pulse into an ultrasonic pressure wave. When coupled to the body, the pressure wave transmits through body tissues focusing on a specific region of interest. The Doppler functions of the system process the Doppler shift frequencies from the echoes of moving targets, such as blood, to detect and graphically display the Doppler shift of these tissues as flow. The Doppler mode creates waves in soft tissues and estimates the tissue stiffness by determining the speed at which these shear waves travel. Stiffness measurements are expressed both in m/s or in kPa (Fig 12). ARFI technique has been validated against VCTE and histology for the staging of fibrosis and seems to give better results compared to VCTE in the detection and grading of PH. In particular, a good correlation was found between SS measurements and the presence and size of varices. More recently real

time 2D shear wave elastography (Aixplorer, Supersonic) showed also a better correlation with liver fibrosis and the presence of clinically significant PH than VCTE [91]. ElastPQ has shown through the years an excellent correlation with VCTE for the assessment of different grades of liver fibrosis [92]. The integration of ultrasound machines with shear wave and point elastography software has clear advantages over VCTE. In the latter, the M-mode shown on the monitor helps to detect the homogeneity of the underlying liver parenchyma providing guidence for a correct measurement acquisition. With pSWE techniques a high definition ultrasound image is visualised, allowing the operator to explore the liver and gain information on the parenchymal appearance, outline, the presence of focal lesions as well as interrogating the portal venous system and measuring spleen size. Very small amount of free fluid can also be detectable and be highlighted as a first subtle sign of clinical decompensation. The measurement of tissue stiffness in addition provides an incomparable diagnostic tool which helps to shed light in cases in which fibrosis and even cirrhosis can be misdiagnosed because the baseline ultrasound findings lack specificity (Fig 13 A, B, C). It also can provide valuable information in more complex cases in which the distribution of fibrosis is particularly patchy such as PSC (Fig 14) in which the segmental distribution of biliary strictures is irregular and can eventually spare the right liver lobe, giving the false impression that the liver is either not affected or only marginally affected by fibrosis. Ultrasound imaging in this case will allow the evaluation of the left lobe and assess its structure (Fig 15 A and B) highlighting the presence of possible abnormalities. Moreover

pSWE can also be used to measure SS, which is particularly important for the detection and grading of PH. Nevertheless there is limited data on the use of ElastPQ for the assessment of SS, and this technique has not yet been validated for the detection, grading and characterisation of CPH and NCPH [93].

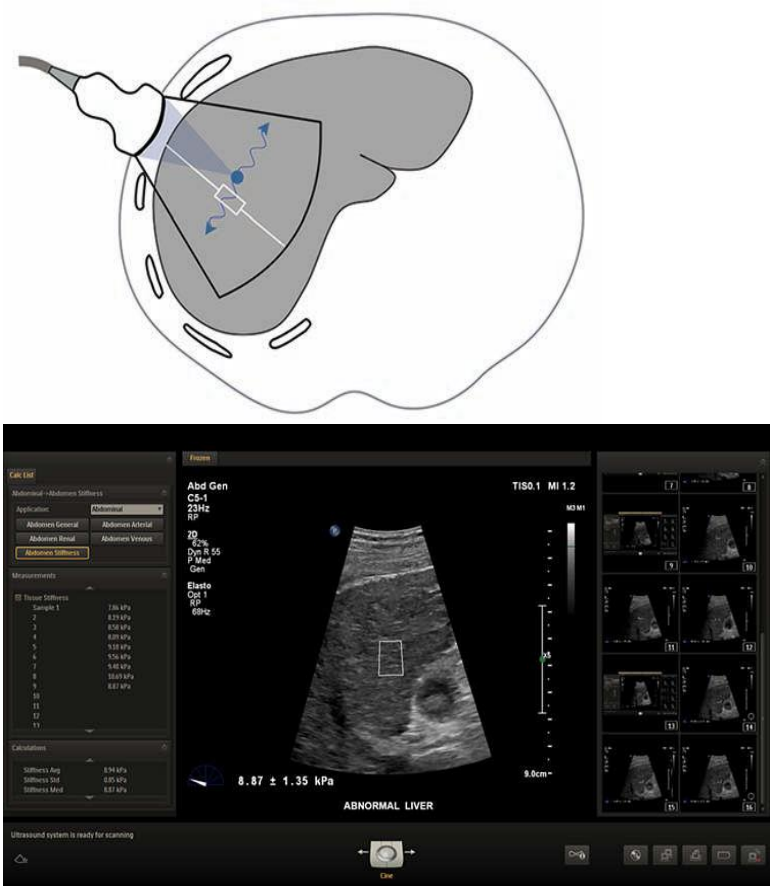

Fig 12. ElastPQ (Point Shear Wave Elastography): a small region of interest of 1 cm x 0.5 cm is placed on the hepatic parenchyma usually 1-2 cm below the liver capsule in order to measure liver stiffness. The shear waves are propagated transversally bypassing the possible limitation of narrow intercostal spaces, the presence of free fluid surrounding the liver and increased subcutaneous fat.

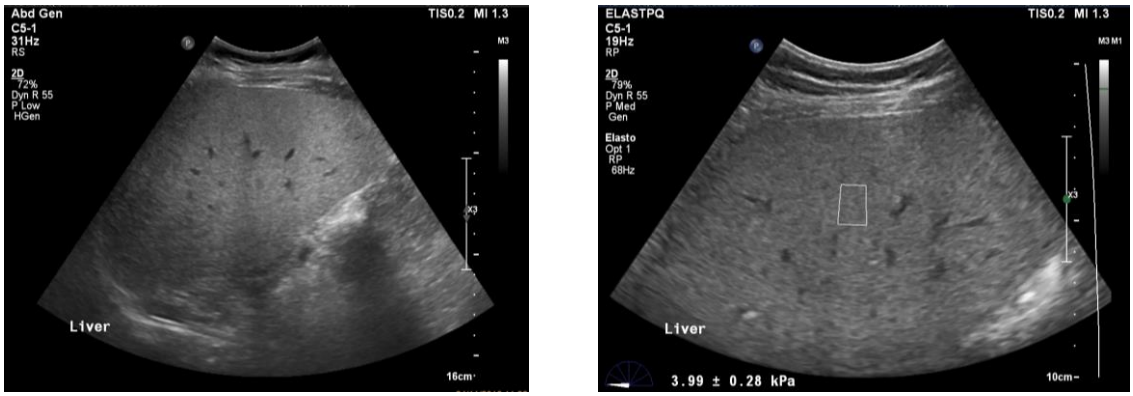

Fig 13 A. Steatotic-looking liver in a patient with mild transaminitis. ElastPQ reveals a normal liver stiffness value of 3.99 kPa, excluding the presence of underlying fibrosis.

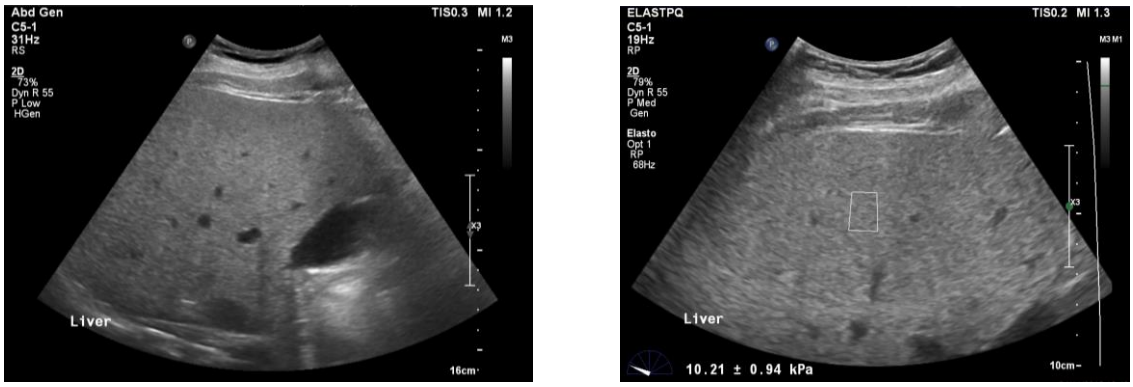

Fig 13 B. Hepatic steatosis with similar appearance. ElastPQ shows a liver stiffness of 10.21 kPa compatible with moderate/severe fibrosis.

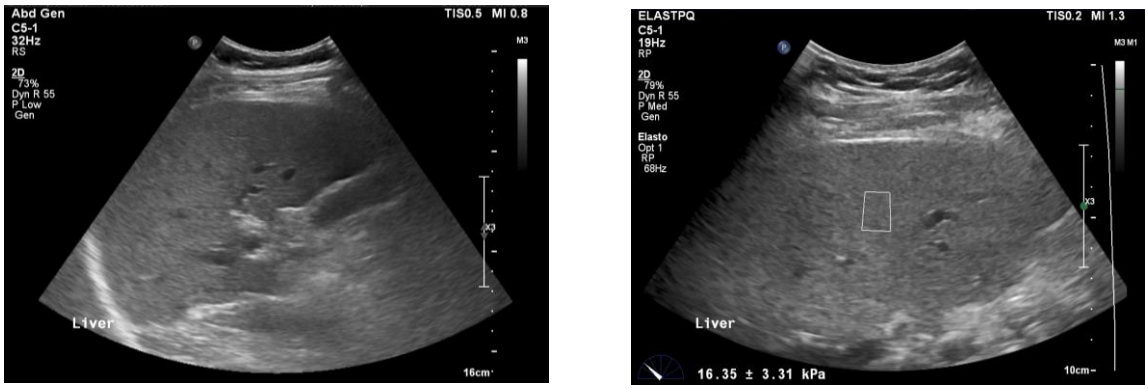

Fig 13 C. Hepatic steatosis with similar B-Mode features to the previous two cases. There is increased parenchymal echogenicity in keeping with hepatic steatosis. The echotexture is homogeneous and the outline is smooth. However liver stiffness is increased measuring 16.35 kPa, which is compatible with cirrhosis. In all three of the above-described cases the baseline ultrasound report highlighted only hepatic steatosis. The presence of underlying fibrosis and especially cirrhosis proven on biopsy was unsuspectable without an elastography assessment. None of the patients had a significant degree of inflammation to justify an increase in stiffness.

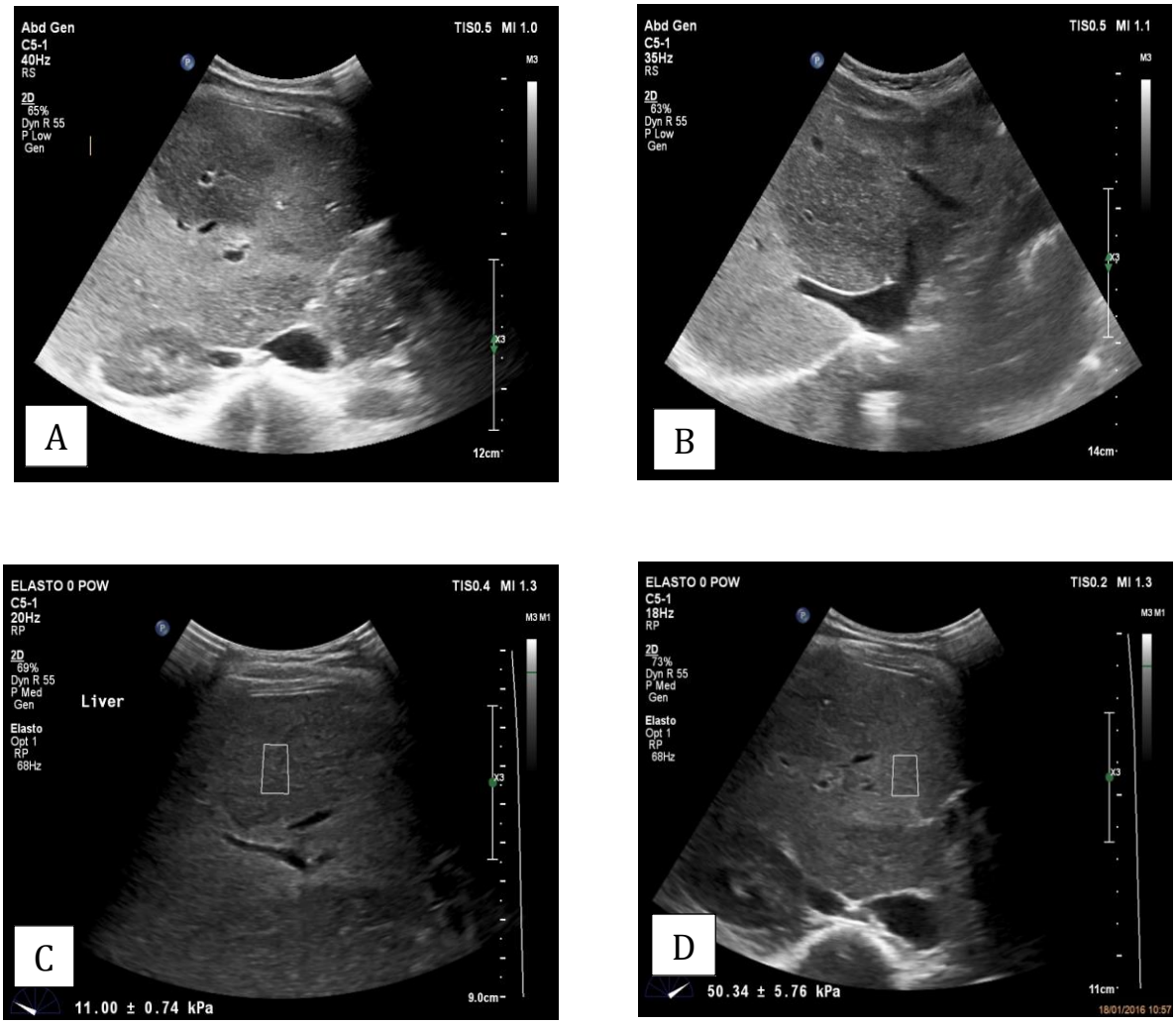

Fig 14. Non-invasive assessment of liver disease in a patient with PSC. The echotexture is quite homogeneous, however the distinctive feature is the presence of well-defined areas of different echogenicity scattered throughout the liver (A and B). The hyperechoic areas were proven to correspond to areas of reduced biliary drainage secondary to biliary thickening and stricturing on MRCP. ElastPQ was used to measure tissue stiffness in the different areas showing that the hypoechoic "dark" areas (C) had a stiffness up to 10-15 kPa and the hyperechoic ("bright") areas (D) were in the range of 50-60 kPa. Segmental distribution of fibrosis is typical in PSC. This case demonstrates how these differences can be identified and "sampled" with ElastPQ.

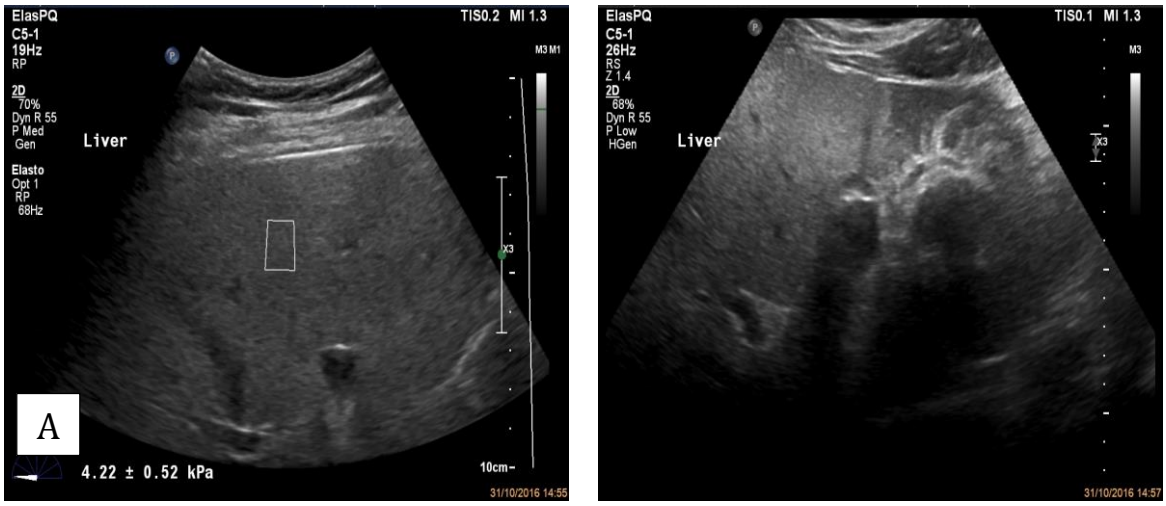

Fig 15 A. Two images from an abdominal scan performed in a patient with PSC. The liver has a steatotic appearance. The right liver is smooth in outline and has a normal stiffness value (4.22 kPa). The left liver lobe is shrunken and the biliary ducts are thickened and dilated. Liver stiffness could not be measured in the left lobe because of technical limitations. The patient had a high BMI and in order to visualize the left lobe increased subcostal pressure was exerted with the probe which would result in over imposed liver stiffness.

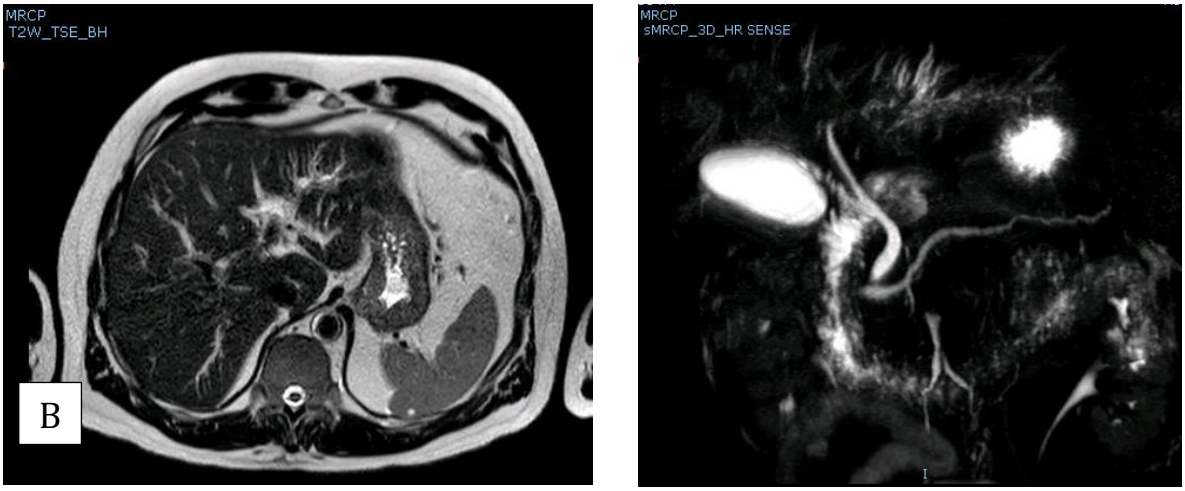

Fig 15 B. MRCP of the same patient showing in detail the left lobe atrophy with thickening and dilatation of the left lobe biliary system.

6. SPLEEN IN CHRONIC LIVER DISEASE AND PH

In order to understand the rationale behind SS measurements and interpret the results appropriately, a brief introduction on splenic function, structure and relative modifications in this clinical context is mandatory.

6.1 SPLENIC FUNCTION AND STRUCTURE

The spleen is the largest lymphoid organ of the human body and has the fundamental role of filtering the blood eleminating possible microbial threats. It has also important hematological and metabolic functions. It receives blood from the splenic artery and is drained by the splenic vein which together with the superior mesenteric vein constitute the portal venous system. The splenic stroma is constituted by white and red pulp and the marginal zone. The white pulp consists predominantly of lymphocytes, together with macrophages and other free cells, lying in a specialized reticular meshwork composed of concentric layers of stromal cells. In normal conditions three quartes of the volume of the human spleen consists of the red pulp, which comprises slender non-anastomosing arterial vessels (penicilli), the splenic cords of Billroth, the venous sinuses and the pulp veins which drain into the splenic veins and hence are in direct communication with the portal vein. The marginal zone is located between the white and red pulp and has fundamental immune and structural functions. Owing to the communication with the portal venous system, in the presence of PH, we can expect an increase in red pulp congestion with increased spleen size.

6.2 **SPLEEN CHARACTERISTICS IN CHRONIC LIVER DISEASE**

In general, considering the anatomical location of the spleen, an increase in portal vascular resistance with consequent reduction in portal blood flow should lead to splenic stagnant flow. However, if splenomegaly would only be the consequence of congestion due to PH, a relationship between splenomegaly and portal pressure would always be expected. Instead, in some cases despite the presence of CSPH, no correlation has been found between spleen size and portal pressure [94-97] or the degree of oesophageal varices [98]. Altogether these data highlight that PH is not the only determinant of splenomegaly in cirrhosis and also that PH is not always associated to increased spleen size. Histopathological studies have demonstrated a clear modification of the splenic architecture in cirrhosis with the presence of diffuse tissue fibrosis and neo-angiogenesis [99-102]. An increase in the white pulp volume has also been highlighted, with an increase in the arterial bed and in peri-arterial lymphatic sheaths [100,103-105]. The increase in white pulp indicates a pronounced immunologic involvement in the genesis of cirrhotic splenomegaly which often can be observed for example in patients with PSC, especially when there is an association with inflammatory bowel disease. A recent retrospective study showed that spleen size differs according to the underlying aetiology of liver disease [106] suggesting that the different splenic compartments are proportionally involved according to the underlying cause and pathophysiological events which differently characterize chronic liver disease. Therefore, splenomegaly in cirrhosis cannot be simply classified as

congestive, but rather as congestive-hyperplastic without forgetting that the inflammatory changes may lead in the long term to the development of a intrasplenic fibrotic component (Fig 16). Along these lines a complete resolution of cirrhotic splenomegaly after liver transplantation has never been reported, probably because of the large irreversibility of the structural changes occurring during the long clinical course of cirrhosis. Nevertheless, although the size does not show any significant change, post-transplant measurements reveal a dramatic fall in SS values, suggesting that this parameter reflects the congestion of the red pulp which is surely expression of the resolution of PH [107].

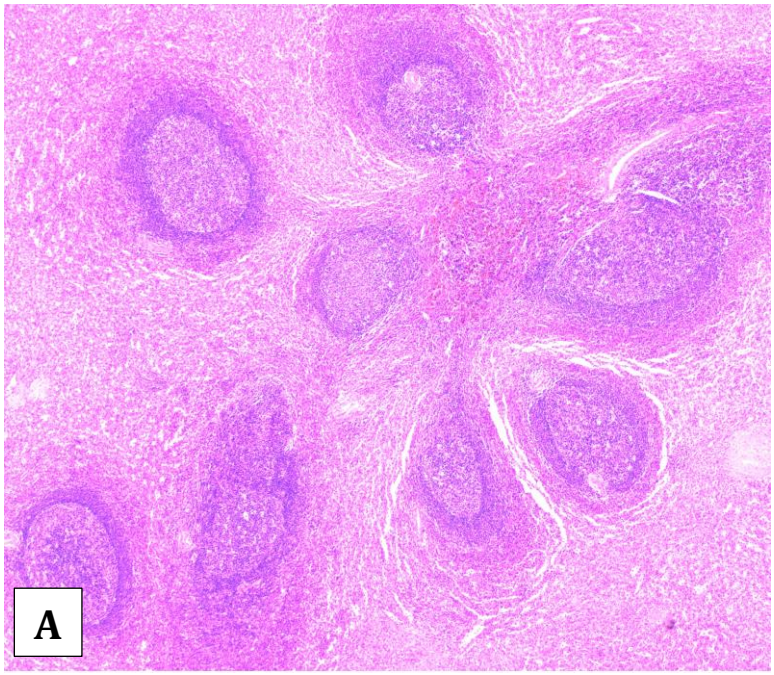

*Fig 16 A. Overview of splenic histology (**A**) in a patient with PSC who underwent splenectomy at the time of liver transplantation.*

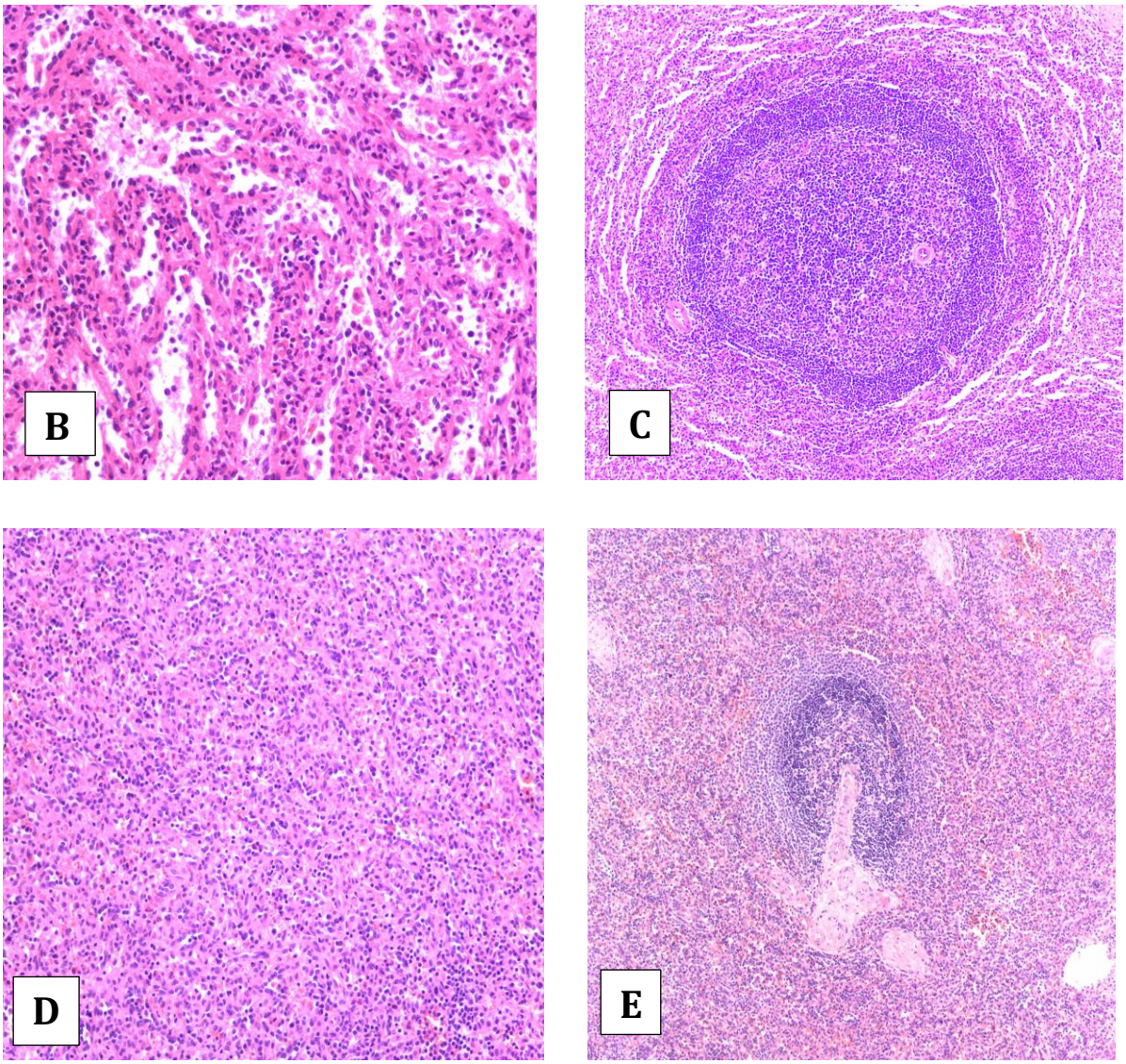

*Fig 16 B,C,D,E. The white areas (best seen in **B**) are the dilated splenic sinusoids which are a consequence of splenic congestion secondary to portal hypertension. There is pronounced lymphoid hyperplasia (**A** and **C**) due to activation of the germinative centres which denotes inflammatory/lymphoid splenic reaction. Between the sinusoids there are areas crossed by thickened fibrotic bands as a consequence of a degree of splenic fibrosis. In comparison (below) we show a normal spleen from a patient who underwent splenectomy because of abdominal trauma. Normal red (**D**) and white splenic pulp (**E**). The red pulp is compact (the sinusoids are virtual spaces in this section and cannot be distinguished) and the lymphoid follicles are small and not activated. The reticulum is so thin that it can barely be noticed.*

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6.3 **SPLENIC-RELATED PARAMETERS IN THE ASSESSMENT OF CIRRHOSIS**

The possibility of predicting the presence of oesophageal varices by using clinical parameters related to splenomegaly was initially suggested with the use of spleen diameter, assessed by ultrasonography, in the platelet count/spleen diameter ratio (Plt/Spl) proposed by Giannini et al. [108-109] and in the score proposed by Kim et al. including also the measurement of LS [110]. Along these lines the study by Berzigotti et al. proposed risk scores for both CSPH and the presence of oesophageal varices, based on the combination of LS, spleen size and platelet count [111]. Stefanescu et al [112] and Colecchia et al [113] proposed and the latter carried out a study on the measurement of SS measured by VTCE for the prediction of esophageal varices in patients with HCV-related cirrhosis. All patients also underwent measurement of HVPG and gastroscopy. The ability of both SS and LS to predict CSPH and the presence of oesophageal varices was compared to the LSPS score, and platelet count to spleen diameter score. SS and LS were more accurate than other non-invasive parameters in identifying patients with oesophageal varices and different degrees of PH. One of the most interesting findings of this study was the presence of a strong direct correlation between SS and the whole range of HVPG values >5 mm Hg, indicating that the increase in SS progresses closely with the progression of PH from the early to the late stages of cirrhosis. These results suggest that, in patients with cirrhosis, SS is possibly characterized by a wider range of application when compared to LS, probably because of a progressively higher relevance of extra-hepatic

factors conditioning the increase of portal pressure [80, 114]. These results were largely confirmed in a similarly designed study performed in 200 patients with cirrhosis due to different aetiologies [115]. The possibility of expanding these studies by employing an alternative method for the measurement of LS and SS, such as ARFI, was suggested by a study where both LS and SS were employed for predicting liver fibrosis stage in patients with HCV- and HBV-related chronic liver disease [116]. Finally Colecchia and co-workers [117] showed that, in compensated cirrhotic patients, a SS and MELD predictive model represents an accurate predictor of clinical decompensation, with an accuracy at least equivalent to that of HVPG.

7. LIVER BIOPSY AND PH IN CHRONIC LIVER DISEASE

Liver fibrosis is part of the structural and functional alterations which typically characterize chronic liver diseases. It has been described as one of the main prognostic factors, as the amount of fibrosis is correlated with the development of cirrhosis, PH and liver-related complications. However, semiquantiative measurements of liver fibrosis have shown poor correlation with the true stage of CLD and hard endpoints such as CSPH, hence they cannot be used for prognostication. Moreover, systems such as Ishak and Metavir are used to describe viral hepatitis-related fibrosis and their employment to quantify/describe fibrosis in other aetiologies is inappropriate. Histological systems to sub-classify cirrhosis have been used, mainly based on semi-quantitative evaluation of nodular size and

septal width [118]. These include proposals by Laennec based on the original histological description of cirrhosis [119], by Nagula [120], Kumar [121] and Sethasine which showed a good correlation with HVPG [122]. Over the last few years computer-aided morphometric measurement of collagen proportional area, a partly automated technique, was developed and validated providing an accurate and linear evaluation of the amount of fibrosis [123]. In fact, quantitative computer-assisted digital-image analysis (DIA) of histological liver sections was proven to be a better histological index than traditional stage scores correlating with all stages of fibrosis and HVPG values in patients with post-transplant HCV infection, moreover predcting liver decompensation at 1 year after OLT [124]. More recently these results were corroborated by other studies which confirmed that CPA is a reliable predictor of the presence of oesophageal varices, hence of CSPH (CPA ≥ 14 %) and also of hepatic decompensation (CPA ≥ 18%) [125]. In conclusion liver histology, assuming there is no sampling error, provides unique diagnostic information particularly for further investigating and distinguishing causes of intrahepatic portal hypertension which may differ from cirrhosis [55]. In addition morphometric analysis measuring CPA should be carried out since it is a true objective measurement of the amount of collagen, it faithfully correlates with HVPG and has prognostic significance.

8. AIMS AND RESEARCH PLAN

78 patients who were admitted to the Royal Free Hospital and underwent haemodynamic assessment with HVPG measurement were prospectively recruited in the study in accordance with the following inclusion and exclusion criteria.

Inclusion Criteria:

- patients with chronic liver disease undergoing HVPG measurement.
- Age > 18

Exclusion Criteria:

- Unable to give consent
- Beta blockers, nitrates and statins

The research study objectives were the following:

1. To evaluate the correlation between VCTE and ElastPQ (pSWE) in a cohort of patients with chronic liver disease
2. To evaluate the correlation of LS and SS measured by ElastPQ with HVPG in patients with chronic liver disease and establish diagnostic cutoffs of SS for diagnosing CSPH
3. To evaluate the correlation between fibrosis grade measured with CPA and LS and SS measured by ElastPQ

4. To assess the accuracy of LS and SS and their ratio for the characterization of PH distinguishing CPH from NCPH.

9. PATIENTS AND METHODS

9.1 METHODOLOGY OF INVASIVE AND NON-INVASIVE MEASUREMENT OF PH

After an overnight fast, an abdominal ultrasound scan was carried out acquiring both LS and SS with ElastPQ. In a subgroup of patients VCTE measurements were also acquired and paired with ElastPQ. An expert radiologist or hepatologist, blinded to the elastography results, carried out the haemodynamic assessment obtaining the mean value from 3 consecutive HVPG measurements. HVPG which was classified in different grades as follows: grade 0 = HVPG 1-5 mmHg NPH (No PH); grade 1 = HVPG 6-9 mmHg (NCSPH); grade 2 = HVPG 10-11 mmHg (CSPH); grade 3 = HVPG ≥ 12 mmHg SPH (Severe PH).

All patients underwent routine blood tests including full blood count, coagulation screen, creatinine, urea, liver function tests (ALT, AST, GGT, ALP, bilirubin and serum albumin).

A subgroup of 41 patients had a histological sample taken at the time of the HVPG measurement by transjugular approach, percutaneous liver biopsy (within a month prior to the haemodynamic assessment) and from the liver

specimen obtained from liver resection for those patients who underwent surgery to remove the tumour (within a month after the haemodynamic assessment).

The histopathological analysis was carried out performing CPA in order to have an objective evaluation of fibrosis and also because CPA is known to correlate well with the severity of PH. To corroborate these findings in our population we first correlated CPA with different grades of HVPG (Fig 18) and then we looked at the correlation of LS and SS using CPA cutoff levels for CSPH as previously described (14%).

Fibrosis scoring systems such as APRI, FIB-4 were compared to both CPA and LS measured with ElastPQ.

A last substudy was finally performed to evaluate the usefulness and accuracy of ElastPQ in distinguishing CPH from NCPH in patients with EHPVO. Patients were diagnosed as having clinically significant NCPH secondary to EHPVO according to radiological (evidence of porto-systemic vascular collaterals shown on contrast CT or MRI) criteria. All patients in the cirrhotic group had a HVPG ≥ 10 mmHg.

9.2 **TECHNICAL ASPECTS OF HVPG MEASUREMENT**

Under ultrasound guidance and with local anesthesia a venous introducer was placed in the right internal jugular vein by Seldinger technique. Under fluoroscopic control and continuous electrocardiographic and arterial pressure monitoring, a 7 French balloon-tipped catheter (Medi-Tech Boston Scientific Cork, Cork, UK) was guided into the right hepatic vein for measurement of WHVP and FHVP. Adequacy of occlusion was checked by injection of 5 ml of iodinated radiological contrast medium (Iopamiro 370, Bracco, Milan, Italy). Three consecutive readings were taken and the mean value acquired as the reference measurement for the diagnosis of portal pressure (Fig 17 A, B).

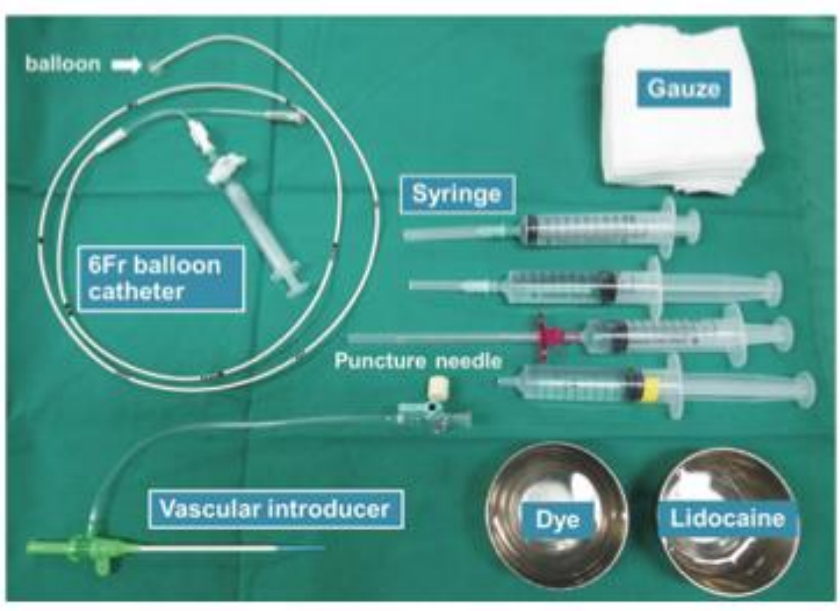


Fig 17 A. The kit used for HVPG measurement includes local anesthesia, normal saline, contrast dye, puncture needle, guide wire, vascular introducer, arrow sheath, cobra catheter, Berenstein balloon catheter and ultrasound probe to guide the puncture of the internal jugular vein [34].

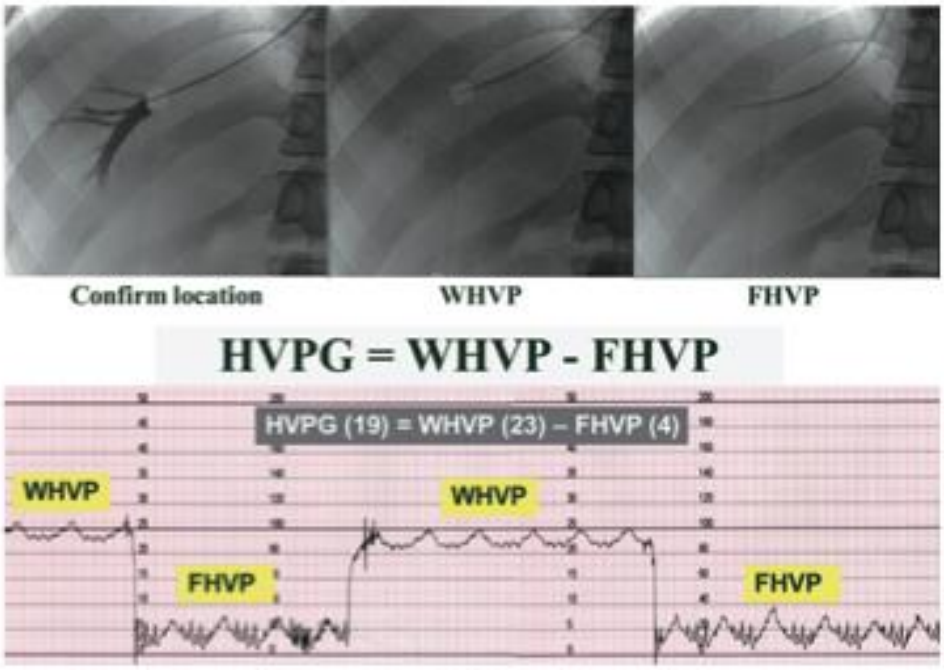


Fig 17 B. A sequence of fluoroscopic images during HVPG measurement shows confirmation of correct location of the catheter in the right hepatic vein. This is done by injecting a small amount of contrast with the balloon catheter inflated. The contrast will be retained distally to the balloon if the position is correct. The balloon is inflated and a wedge pressure is measure. Deflating the balloon will allow to obtain a free hepatic venous pressure. In the other figure a pressure trace shows the corresponding HVPG measurements [34].

9.3 **HISTOPATHOLOGY ASSESSMENT: COLLAGEN PROPORTIONATE AREA**

Computer-assisted digital image analysis (DIA) of histological sections, histochemically stained by the picroSirius red technique, is a method for measuring fibrosis morphologically. PicroSirius red staining identifies tissue collagen primarily.

The quantity of bound stain correlates with chemically determined collagen content and morphometrically determined hepatic fibrosis. Digital image analysis uses segmentation of digital images to measure the area of collagen and of tissue, producing a “fibrosis ratio” CPA [124].

Liver histology was obtained either at the same time as HVPG measurement by a transjugualr approach, or by percutaneous liver biopsy (not more than 3 months from HVPG measurement), or by tissue sampling obtained with liver resections for those patients who underwent surgery for HCC (within 4 weeks from the haemodynamic assessment). The sections of each biopsy stained with PicroSirius red were used for digital image analysis (DIA), which was performed by an expert pathologist (A. Hall). The equipment setup used consisted of a digital camera (Canon Powershot A640 attached to a close-up copystand with backlighting) connected to a compatible personal computer. After whole section digital image capture, CPA was measured with Zeiss KS300 image analysis software. The CPA measurement included editing steps to eliminate image artifacts and structural collagen in large portal tracts and blood vessel walls (which do not represent disease-related liver fibrosis). Unfilled natural spaces such as vascular cavities were not included in the measurements and, because non-collagenous cellular areas such as lymphoid aggregates in portal tracts are not stained (red) with picroSirius red, these also were not included [118] (Fig 17).

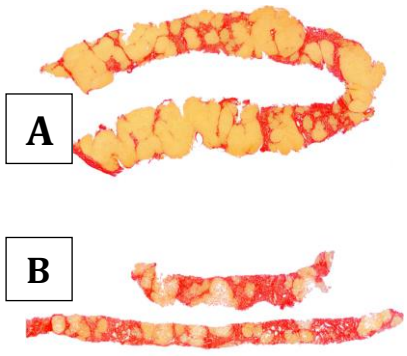


Fig 18. Histopathology sections of two patients with cirrhosis. No other known method of histological analysis was able to distinguish these two samples. CPA was 21% in A and 46% in B. The latter was associated to severe portal hypertension and clinical decompensation [119].

9.4 **ELASTOGRAPHY ASSESSMENT**

9.4.1 **ElastPQ**

The elastography measurements were performed using an Affiniti 70 G ultrasound system (Philips Healthcare, Bothell, WA, United States) with a convex broadband probe C5-1 and ElastPQ® software. As with other shear wave elastography methods, Point Shear Wave Elastography is a technique in which shear waves are generated inside the liver using radiation force from a focused ultrasound beam. The ultrasound machine monitors the shear wave propagation using a Doppler-like ultrasound technique, and measures the velocity of the shear wave. The shear wave velocity is displayed in meters per second (m/s) or in kPa through Young's modulus $E = 3 (vS2.\rho)$, where E is Young's modulus, vS is the shear wave velocity and ρ is the density of the tissue.

Both liver and spleen measurements were carried out with the patient lying supine and with both arms in maximal abduction. The liver was imaged and the region of interest was placed 2 cm below the liver capsule, in perpendicular position compared to the liver surface and away from vessels, ligaments or biliary ducts. A total of 10 measurements were taken and median value and standard deviation were acquired. A measurement was considered accurate in the presence of a standard deviation lower than 30% of the median value. Subsequently the spleen was imaged through the left intercostal spaces, and keeping the region of interest 1-2 cm below the splenic capsule and away from large vessels. The region of interest was placed above the splenic hilum at the level of the mid splenic pole. When

the spleen was normal in size or not particularly enlarged, or the mid pole was not entirely visible, the lower pole was sampled. 10 consecutive measurements were taken and median and standard deviation were acquired. Both longitudinal diameter and area were also measured.

9.4.2 **VCTE**

The patient was positioned supine with the right arm in maximal abduction. After having placed a small amount of gel on the tip of the transducer, this was placed between the intercostal spaces and 10 consecutive measurements were acquired. The measurements were considered accurate in the presence of an IQR <30% and a success rate higher than 60%. Since part of the population undergoing HVPG was affected by liver cancer, a preliminary ultrasound scan was always carried out before fibroscan measurements to avoid the presence of an underlying lesion, or to know if transient elastography was feasible or not because of inaccessible healthy liver parenchyma.

10. **DATA AND STATISTICAL ANALYSIS**

Statistical analysis was performed by using the Statistical Package for Social Science (SPSS), release 20.0 and a p-value <0.05 was considered statistically significant. All data were first analysed for normality of distribution using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Data with normal distribution were expressed as mean ± standard deviation (SD); data of skew distribution as median with interquartile rate (IQR), and

frequencies as percentages. On the univariate analysis two or more population medians were compared using the Mann-Whitney or Kruskal-Wallis non-parametric U-test, respectively, two or more population means using T-Student's test or ANOVA. The comparison of categorical variables was carried out using χ^2-test. The correlation of continuous variables was assessed with the Pearson's or Spearman's correlation coefficient if the variables had a normal or non-normal distribution, respectively. A multivariate analysis with a binary logistic regression was performed to identify predictor factors which were expressed as odds ratio (OR) and 95% confident interval (CI). AUROC curves were used to define cut-off values. Cross tabs were used to define specificity, sensitivity, negative predictive value and positive predictive value.

11. RESULTS

11.1 PATIENTS' CHARACTERISTICS

78 patients admitted to the Royal Free Hospital between 2015 and 2016 who underwent haemodynamic assessment with HVPG measurement were recruited in this study. 8 patients were excluded because of technical difficulties. In 6 of these patients the spleen was too small leading to high standard deviation, hence these were considered not reliable and excluded from the study. One patient had undergone splenectomy and another had large volume ascites and hyperdynamic circulation leading to subtle movements of the spleen within the ascitic fluid and inaccurate readings.

70 patients (55 male; 15 female) of mixed aetiolgies (NASH, HCV, HBV, ALD and Other (HCV/ALD, HBV/ALD, HIV/HBV, NASH/HCV, NASH/ALD, PSC/AIH, PBC, AIH, Post OLT/HCV)) were finally enrolled. The population characteristics are described in Table 2.

Gender (male/total)	55/70
Age, (y)	60 ± 12
NASH, n/tot (%)	22/70 (31.4)
HBV, n/tot (%)	6/70 (8.6)
HCV, n/tot (%)	12/70 (17.1)
ALD, n/tot (%)	4/70 (5.7)
OTHER, n/tot (%)	26/70 (37.2)
NPH, n/tot (%)	21/70 (30)
NCSPH, n/tot (%)	23/70 (32.9)
CSPH, n/tot (%)	8/70 (11.4)
SPH, n/tot (%)	18/70 (25.7)
HVPG, mmHg	8.2 ± 4.8
LpSWE (kPa)	14.8 (13.2)
SpSWE (kPa)	40.1 (29.8)
Spleen Diameter (cm)	13 ± 2.9
Spleen Area (cm^2)	50 (38)
Platelets	181 ± 88.6
ALT, UI/l	42.5 (30)
AST, UI/l	39 (31)
ALP, UI/l	91 (56)
Bilirubin, mmol/l	11.50 (12)
Albumin, mg/dl	41.27 ± 5.8
INR	1.04 ± 0.2
Creatinine, mmol/l	72.1 ± 24.5
APRI	0.7 (0.9)
FIB-4	2.6 (2.1)
MELD	7.5 (3)
LSPS	1.2 (2.2)
SSPSA	13.1 (25.4)

*TABLE 2: Description of the study population. NASH (non-alcoholic steatohepatitis); HCV (hepatitis C virus); HBV (hepatitis B virus); ALD (alcohol liver disease); Combined (mixed aetiologies such as HCV/ALD, NASH/HCV HBV/ALD, NASH/ALD, HIV/HBV); Other (Isolated aetiologies such as autoimmune hepatitis, primary biliary cirrhosis, post-transplant patients); NPH (No Portal Hypertension); NCSPH (non-Clinically significant portal hypertension); CSPH (Clinically significant portal hypertension); SPH (Severe Portal Hypertension); HVPG (hepatic venous pressure gradient); LpSWE (Liver Point Shear Wave Elastography); SpSWE (Spleen Point Shear Wave Elastography); ALT (Alanin Transaminase); AST (Aspartate Transaminase). ALP (Alkaline Phosphatase); APRI (AST to platelet ratio score); FIB-4 (Fibrosis 4); MELD (Model for End Stage Liver Disease); LSPS (Liver Stiffness*Spleen Diameter/Platelet Count); SSPSA (Spleen Stiffness X Spleen Area/Platelet Count). Continuous variables are reported as mean ± standard deviation if the distribution is normal; whereas are reported as median (IQR) if they are not normal.*

11.2 **CONCORDANCE BETWEEN VCTE AND ELASTPQ**

45/70 patients had both ElastPQ and fibroscan measurements. We could not perform VCTE in every patient because of logistical reasons due to time limitation, or because it was not feasible (patients affected by a large lesion in the right liver lobe). Of the 45 patients who received both, VCTE was performed the same day prior to HVPG in 34/45 patients and in 11/45 patients the results from a previous fibroscan performed within 3 months were used. Biochemical analysis and clinical status in the latter case did not show any significant difference. ElastPQ showed an excellent correlation with fibroscan (Spearman's 0.941, p <0.0001) (Fig 19).

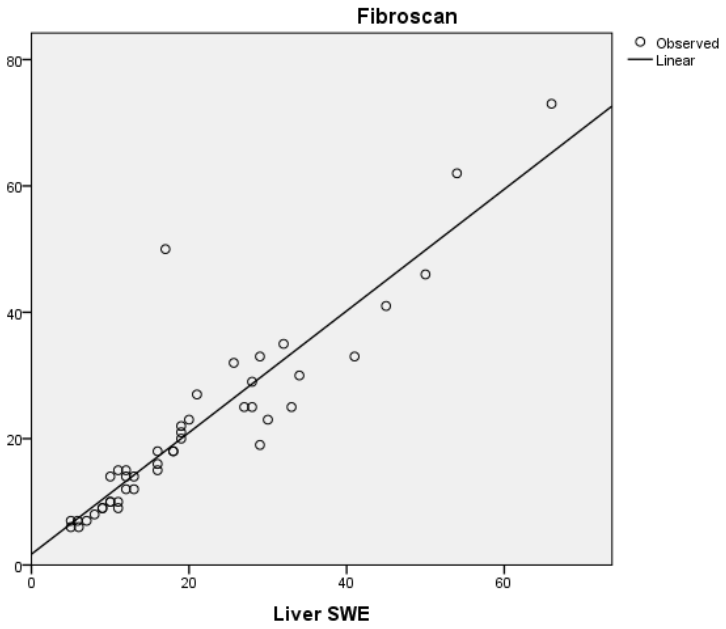


Fig 19. 45/70 patients underwent also VCTE measurement to evaluate its correlation with ElastPQ. A strong correlation was found between the two techniques (Spearman's 0.941;p<0,0001).

11.3 **CORRELATION OF ELASTPQ WITH LIVER FIBROSIS**

41/70 patients had an available histological sample that was compared to LS measured by pSWE. A significant correlation was seen between CPA and each grade of PH (Fig 20). Both liver pSWE ($p<0.0001$) (Fig 21A) and spleen pSWE ($p<0.005$) (Fig 21B) correlated significantly with CPA. These results confirmed once again that CPA reflects the hemodynamic modifications of advanced liver disease and, for the first time that LS and SS measured by ElastPQ correlate significantly with CPA and, hence with PH indirectly measured by the amount of collagen in different categories, and independently from liver disease aetiology. Both CPA and LS correlated significantly with APRI ($p<0.0001$) and FIB-4 ($p <0.002$).

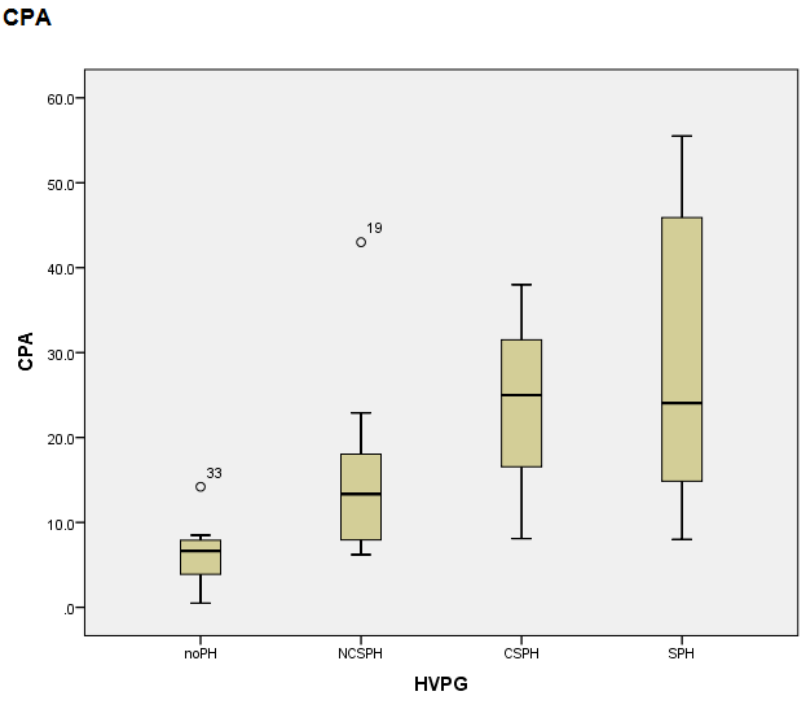
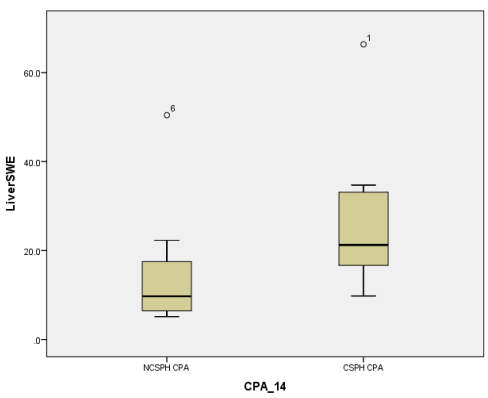


Fig 20. A strong correlation was observed between CPA and HVPG across all categories of portal hypertension (p<0.0001).



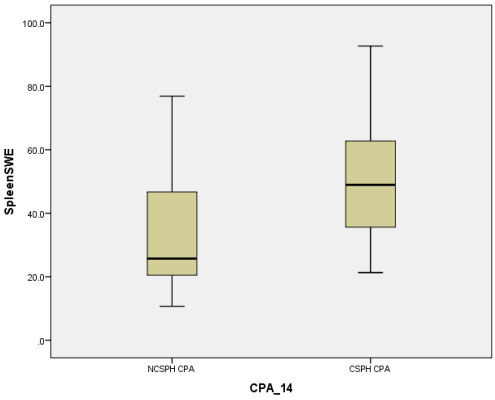


Fig 21A. Liver pSWE and CPA p<0.0001 *Fig 21B . Spleen pSWE and CPA p<0.005*

11.4 DIAGNOSIS AND STAGING OF PH BY ELASTPQ

LS and SS measured by ElastPQ correlated significantly with all categories of PH (Fig 22A and 22B). In particular, on univariate analysis liver pSWE, spleen pSWE, spleen diameter, spleen area, platelet count, LSPS and a new proposed scoring system named SSPSA [Spleen stiffness* (Spleen area/platelet count)] (Fig 23-25) as well as APRI, FIB-4 and MELD, all correlated significantly with HVPG. A significant correlation was also found between PH and bilirubin, ALP, and Albumin. No difference was found concerning age, gender, aetiology, AST, ALT and INR (details in Table 3). On multivariate analysis SS was the only variable that correlated independently with HVPG (OR 1.099; CI 1.017-1.188; p<0.017). LS and SS AUROCs were calculated in order to define relative cutoff values for each category of HVPG (Fig 26-28). The most accurate values were obtained for SS related to CSPH. SS AUROC for HVPG≥10 mmHg was 0.918, p<0.0001, cut off value 42.7 kPa, sensitivity 96%, specificity 84%, negative predictive

value (NPV) 97.4% and positive predictive value (PPV) 78.1%. Details of each category related to both LS and SS are reported in Table 4.

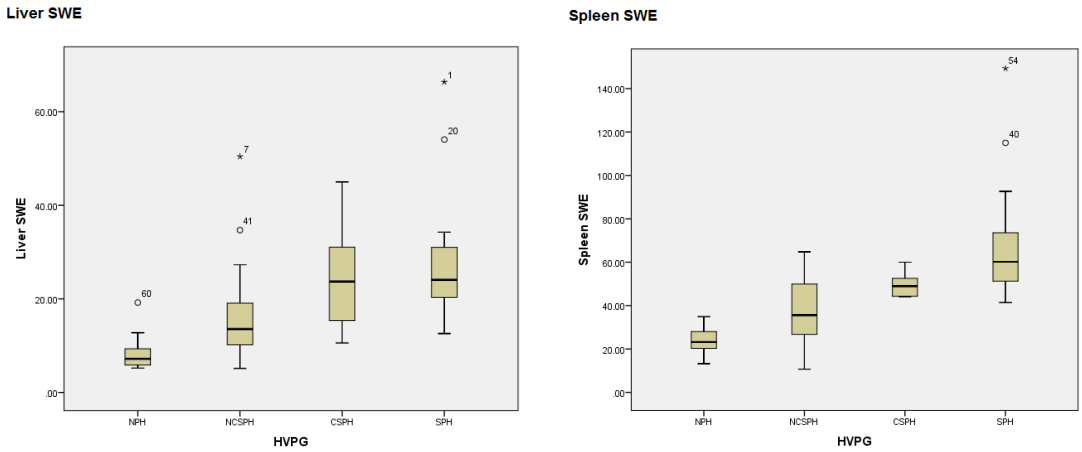


Fig 22A. Liver pSWE correlates significantly across different classes of HVPG. Spleen pSWE shows even a better correlation and a more distinct categorization of the different classes as shown in the graph on the left and below.

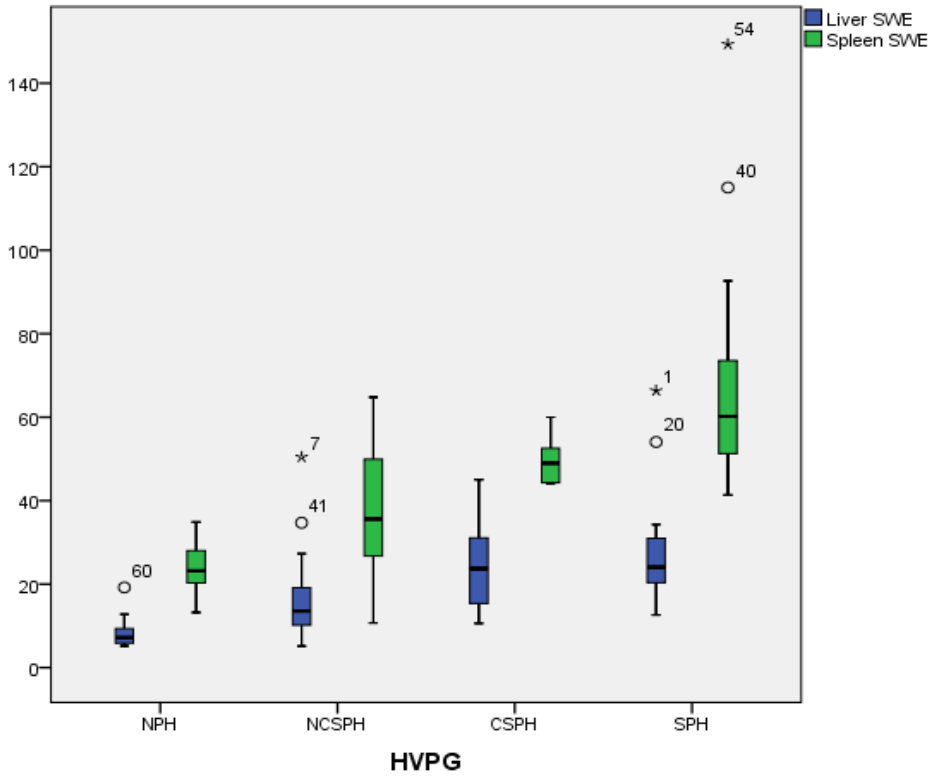


Fig 22B. This figure highlights the differences between the correlations of liver and spleen pSWE with HVPG

	UNIVARIATE ANALYSIS			MULTIVARIATE ANALYSIS		
	NCPH	CSPH	p value	OR	95% CI	p value
AGE (y)	60.8	58.1	NS			
Gender (male/total)	35/44	20/44	NS			
HCV	5	7	NS			
HBV	5	1	NS			
NASH	16	6	NS			
ALD	2	2	NS			
Other	16	10	NS			
AST	34	41.5	NS			
ALT	45.5	35	NS			
Bilirubin	10.5	15.5	<0.012			
ALP	85.5	110	<0.042			
ALB	43	40	<0.005			
Platelets	204.5	125.5	<0.0001			
INR	1	1	NS			
MELD	7	9	<0.012			
APRI	0.4	1.04	<0.001			
FIB-4	1.8	3.2	<0.0001			
LS (pSWE)	9.8	24	<0.0001			
SS (pSWE)	27.9	55.6	<0.0001	1.099	1.017-1.188	<0.017
Spleen Size	12.1	14.5	<0.0001			
Spleen Area	44	72.5	<0.0001			
LSPS	0.63	2.99	<0.0001			
SSPSA	6.5	26.5	<0.0001			

Table 3. Univariate and multivariate analysis of the study population variables compared to HVPG measurement. SS was the only factor independently correlated with HVPG.

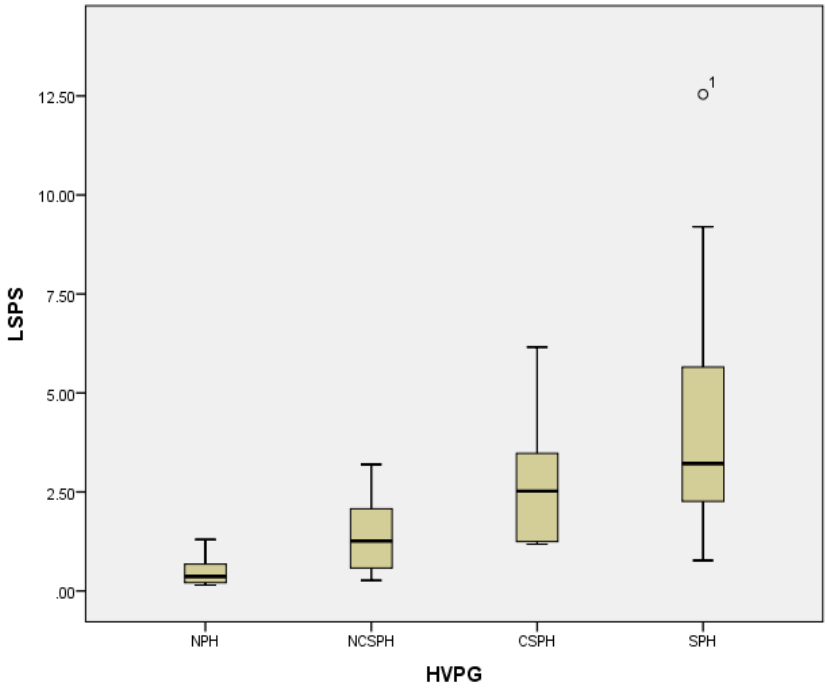


Fig 23. LSPS which is known to have an excellent correlation with HVPG, being able to accurately predict the presence of oesophageal varices, shows a good correlation in our study population across different categories of HVPG (p<0.0001).

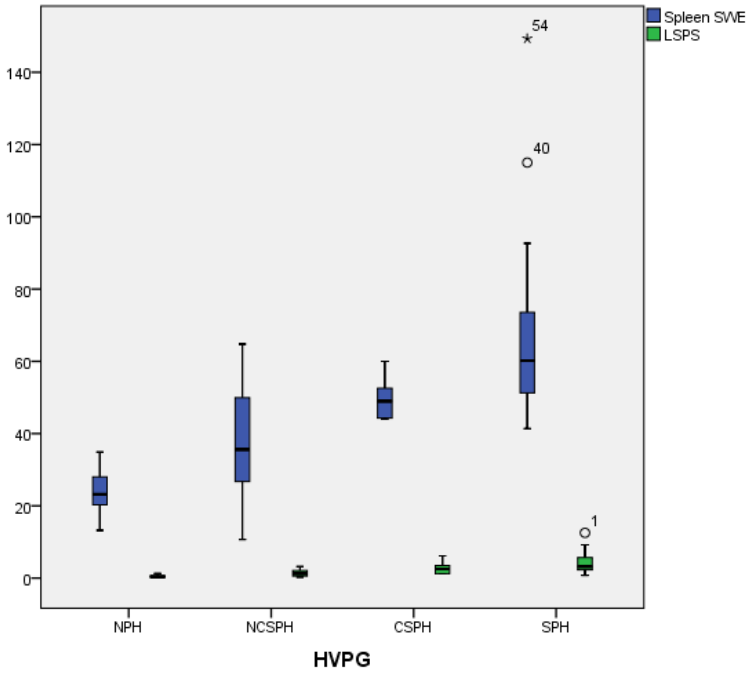


Fig 24. Spleen pSWE and LSPS are compared in this graph. They both correlate significantly with HVPG. However spleen pSWE shows a better and distinct categorization in relation to HVPG classes.

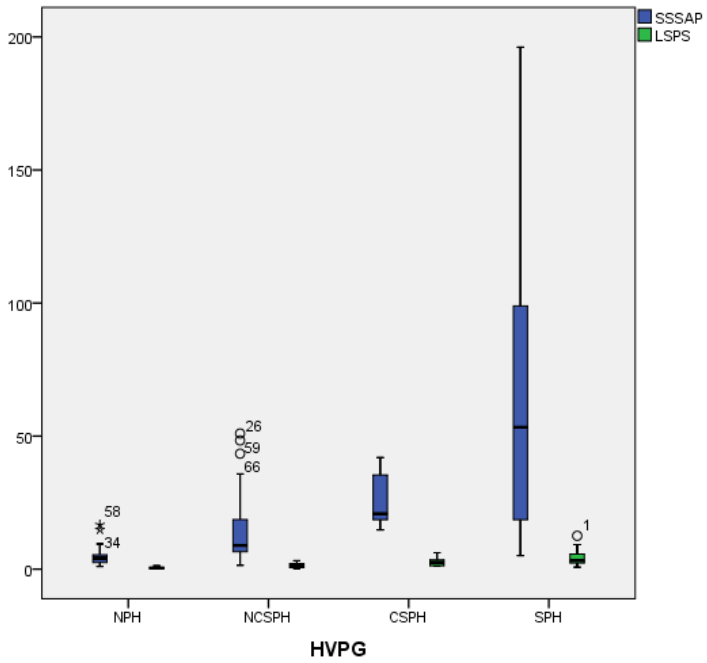


Fig 25. To further increase the accuracy of SS we proposed a new scoring system which included spleen stiffness, spleen area and platelet count (SSPSA). There was a good correlation between SSPSA and all categories of HVPG. The correlation was equal. Both had a p<0.0001. However LSPS AUROC was slightly better (see related AUROC graphs).

LSWE	PORTAL PRESSURE		
	HVPG 6-9	**HVPG 10-11**	**HVPG ≥12**
AUC 95% CI	0.918 (0.851-0.986)	0.895 (0.820-0.970)	0.869 (0.785-0.952)
Cut-off (kPa)	11.8	18.6	19.9
Sensitivity (%)	82	85	87
Specificity (%)	91	84	83
PPV (%)	81.6	84.6	89
NPV (%)	91	84.1	83

SSWE	PORTAL PRESSURE		
	HVPG 6-9	**HVPG 10-11**	**HVPG ≥12**
AUC 95% CI	0.898 (0.825-0.971)	0.918 (0.853-0.983)	0.922 (0.861-0.983)
Cut-off (kPa)	30.2	42.7	50
Sensitivity (%)	84	96	87
Specificity (%)	86	84	83
PPV (%)	83.7	78.1	89
NPV (%)	85.7	97.4	83

Table 4. Results of the AUROCs for both liver and spleen stiffness for different grades of portal hypertension.

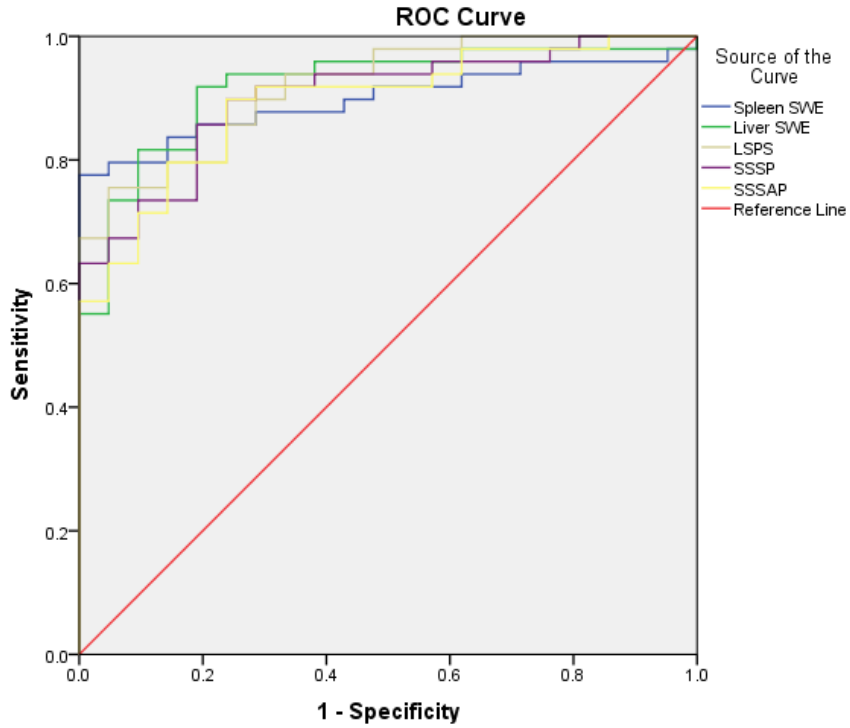


Fig 26. AUROC of LSWE, SSWE, LSPS and SSPSA for NCSPH. SSWE 0.898, LSWE 0.918, LSPS 0.918, SSPSA 0.893.

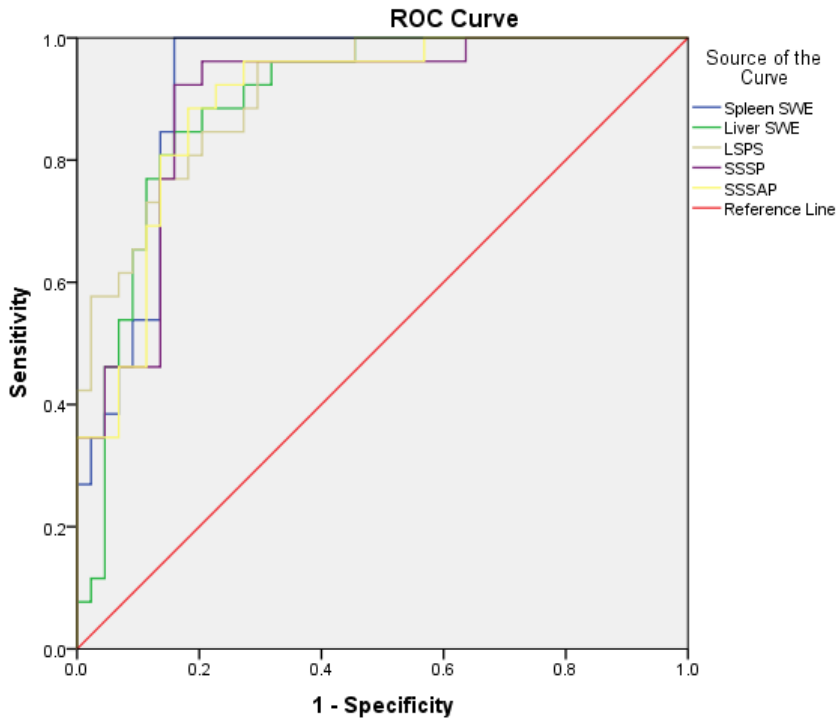


Fig 27. AUROC of LSWE, SSWE, LSPS and SSPSA for CSPH (SSWE 0.918, LSWE 0.895, LSPS 0.911, SSPSA 0.895).

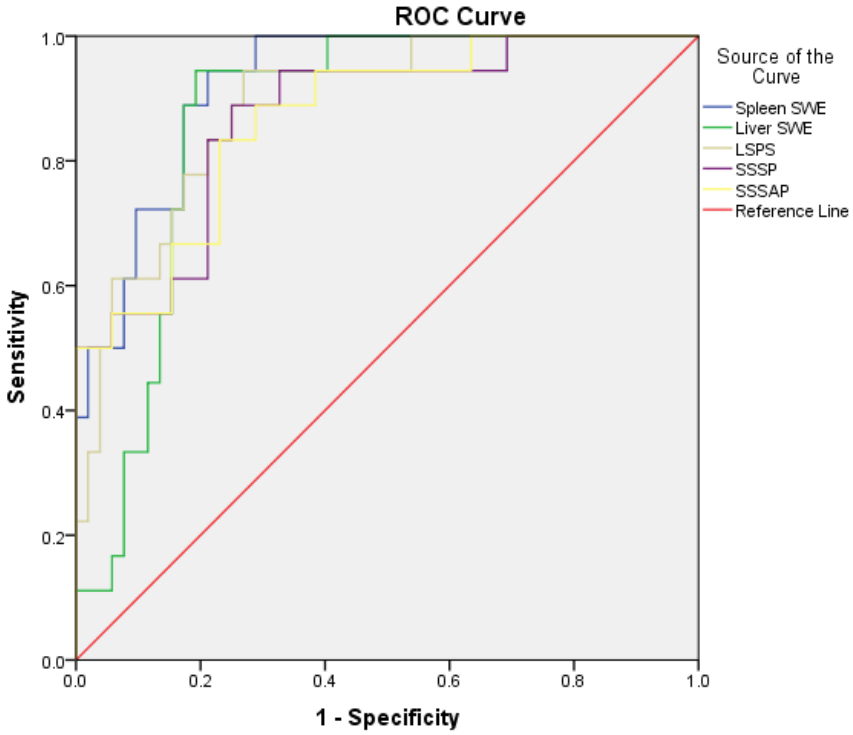


Fig 28. AUROC of LSWE, SSWE, LSPS and SSPSA for SPH (SSWE 0.922, LSWE 0.869, LSPS 0.889, SSPSA 0.869).

11.5 **CHARACTERIZATION OF PH: LS AND SS IN CPH AND NCPH**

Finally we carried out a comparison between a subgroup of patients (26) with CSPH (HVPG ≥ 10 mmHg) and a population of patients (21) with NCPH due to EHPVO. 19/21 had developed PVT secondary to JAK2+ related-thrombophilia and 2 patients had developed PVT as a consequence of pylephlebitis. Liver pSWE, spleen pSWE, splenic area (SA) and the ratio of SS to LS (SS/LS) were all found to have statistically significant differences and were able to distinguish the two groups of patients with PH ($p<0.0001$) (Fig 29-32 and Table 5). In particular the median value of LS in patients with NCPH was normal (<7 kPa) and the SS was almost twice as high as the SS value in cirrhotic patients. Hence the most reliable

parameter to characterize PH is the SS/LS. Figures 33-35 highlight on imaging three examples of the differences between the two groups and the correlations of LS and SS with HVPG in two cirrhotic patients.

	CPH	NCPH	p value
LSWE (kPa)	24	5.8	<0.0001
SSWE (kPa)	55.6	93.3	<0.0001
SA (cm^2)	72.4	116	<0.009
Platelets/mm^3	124	180	<0.001
SS/LS (kPa)	2.3	14.2	<0.0001

Table 5. Results of comparison between CPH and NCPH. All variables were significantly correlated and able to distinguish between the two groups.

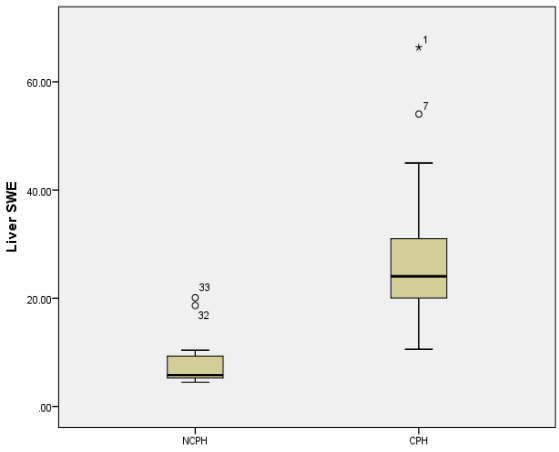


Fig 29. Liver pSWE in CPH and NCPH (p<0.0001).

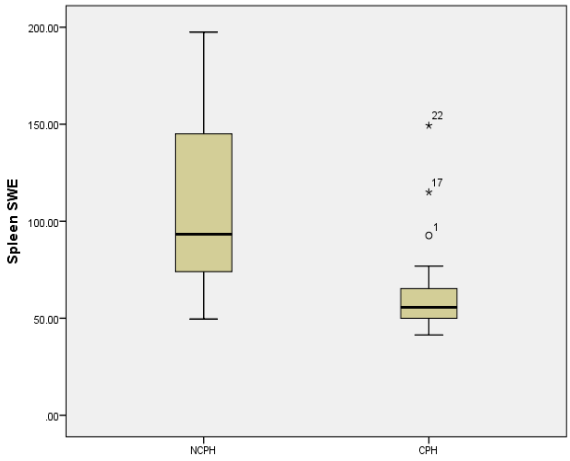


Fig 30. Spleen pSWE in CPH and NCPH

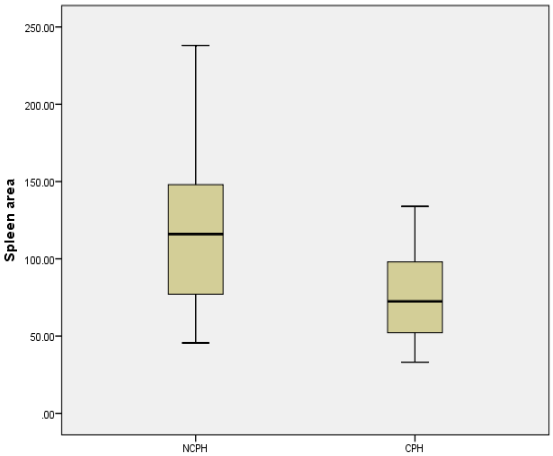


Fig 31. Spleen Area in CPH and NCPH (p<0.009)

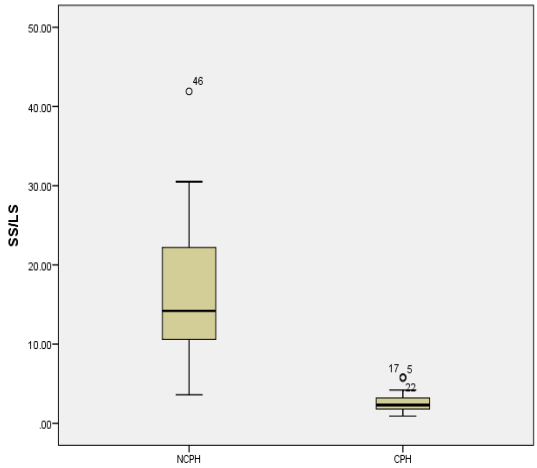


Fig 32. SS/LS in CPH and NCPH (p<0.0001)

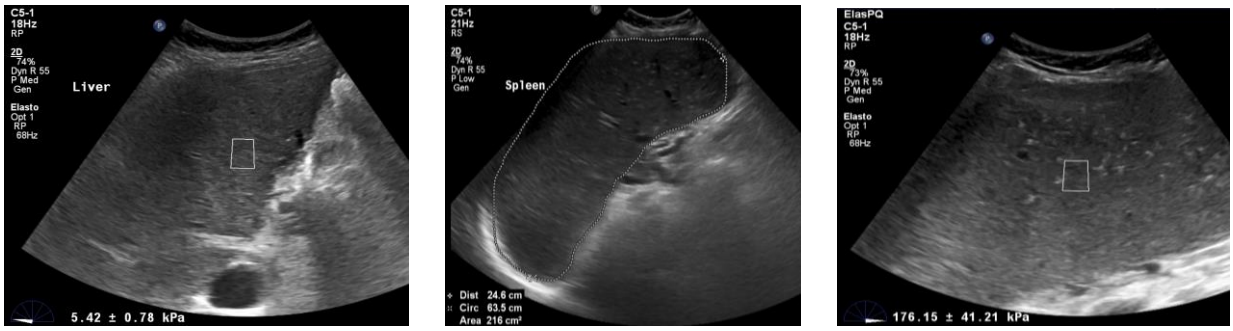


Fig 33. Patient with policytemia vera JAK 2 + and portal vein thrombosis. Massive splenomegaly (spleen diameter 24.6 cm, spleen area 216 cm$^{2.}$). ElastPQ revealed a LS 5.42 kPa and a SS of 176.15 kPa.

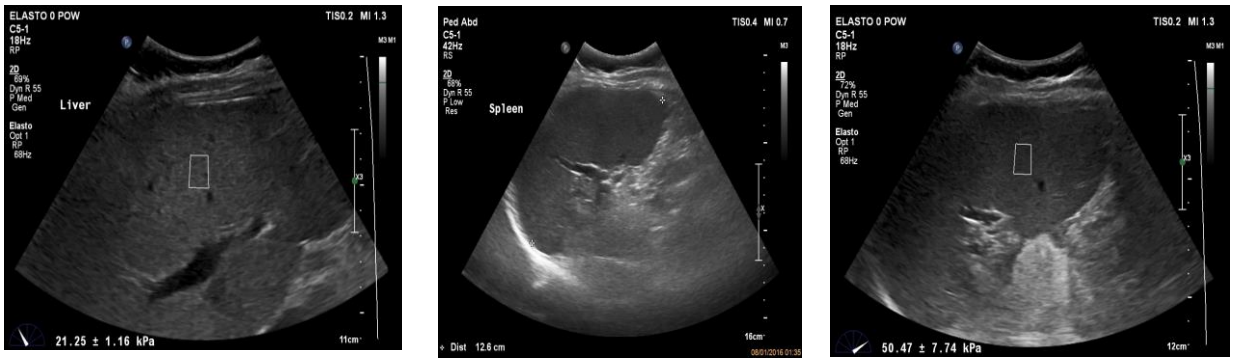


Fig 34. Patient with HCV/HIV related cirrhosis. The outline is smooth the echotexture is homogeneous. The spleen is just slightly enlarged measuring 12.6 cm in diameter. ElastPQ revealed a liver stiffness of 21.25 kPa and a spleen stiffness of 50.47 kPa. HVPG was 12 mmHg.

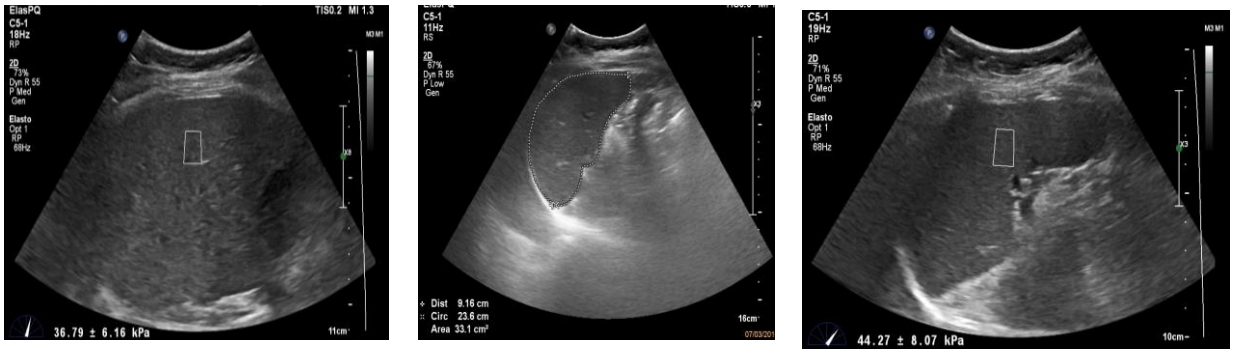


Fig 35. Patient with HCV related cirrhosis. The patient had a small lesion in segment VII not shown in these images and needed to have preoperative assessment in view of liver resection. ElastPQ revealed a LS of 36.7 kPa. The spleen was normal in size measuring 9.16 cm in diameter with an area of 33.1 cm^2, SS was 44.2 kPa and HVPG was 11 mmHg.

12. **DISCUSSION**

More than 60 years have passed since clinicians started investigating the haemodynamic changes of the portal venous system during the course of liver disease and other pathologies. The development of new technologies has provided useful tools to further understand and manage liver disease. The results of this work highlight the practical applications of non-invasive assessment following those pathophysiological discoveries.

The first important finding is that liver stiffness measured with ElastPQ had an excellent correlation with VCTE (Spearman's 0.941, $p<0.0001$) proving that despite the heterogeneity of the population in terms of liver disease aetiology, ElastPQ was able to reproduce faithfully VCTE results. The second finding is the relationship between collagen content and PH. It had already been proven that CPA correlates to HVPG but this was shown mainly in patients with HCV and patients with recurrent HCV infection and rapid progression of liver fibrosis after liver transplantation. Our population was composed of 70 patients with different liver disease aetiologies and different stages of liver disease. CPA was calculated in 41 patients with available histopathological samples. Ideally the analysis should be carried out in distinct subgroups according to the specific aetiologiy but the sample was too small to obtain meaningful results and we could not divide the population. Hence the analysis was carried out on the whole group with no distinctions. Nevertheless this approach gave interesting findings. Although there are known histopathological

differences between different aetiologies such as micro and macronodularity, and amount of collagen and its distribution, CPA correlated very well with each category of HVPG regardless of aetiology. Moreover LS measured by ElastPQ showed also a very good correlation with CPA, and fibrosis related scoring systems such as APRI and FIB-4. CPA is the most reliable histological measurement of liver fibrosis and correlates with PH and has prognostic value. Along these lines we showed that a correlation, although slightly weaker, exists also between CPA and SS highlighting the importance of the latter as a non-invasive parameter with prognostic significance related to a non-invasive assessment of fibrosis and PH. This interconnection is interesting and important in consideration that CPA correlated to HVPG in all different grades and that SS also followed CPA accordingly. This finding has never been described previously.

The results proved the presence of a significant correlation of both LS and SS measured by ElastPQ with HVPG across all categories in the whole population. The AUROCs showed that for patients with NCSPH (HVPG 6-9 mmHg) LS had a better AUROC (AUROC 0.918, cutoff 11.8 kPa, PPV 81.6%, NPV 91%) compared to SS (AUROC 0.898, cut off 30.2 kPa, PPV 83.7%, NPV 85.7%). Nevertheless, with the increase of portal pressure the AUROC of LS decreased progressively while the accuracy of SS instead increased showing the best cutoff for CSPH. The AUROC for SS increased even more for the HVPG category of severe portal hypertension but accuracy was slightly lower. SS performed better than any other variable and was independently correlated to HVPG. This result highlights two important findings. First that SS is the best non-invasive parameter to diagnose CSPH.

In fact, none of the other parameters including LSPS was able to perform as wells as SS. In the attempt to increase further the accuracy of SS a new scoring system composed by a combination of SS, spleen area (which we believe reflects more faithfully spleen size) and platelet count [spleen stiffness*(spleen area/platelet count)] which we named SSPSA was proposed. However, although it significantly correlated with all HVPG categories ($p<0.0001$) it lost its significance on multivariate analysis and had a lower AUROC compared to SS alone. We believe that the reason for this result is to be found in the discrepancy between spleen size and PH. While for NCSPH we obviously expect to find normal sized spleens (in liver disease) we assume in theory to see a proportional increase in size following the raise in portal pressure. However, although spleen diameter and area both showed a very good correlation on univariate analysis ($p<0.0001$) a very high standard deviation was found in patients with CSPH and SPH meaning that this population of patients regardless of the severity of PH could be found to have a very large spleen but also a relatively small one. In view of optimising non-invasive assessment of liver disease this finding is of crucial importance since spleen size and any scoring system that includes it, potentially will reduce their accuracy especially in the presence of CSPH. In fact, it is possible that one of the reasons of this finding is the variety of aetiologies from which our population is composed. As previously mentioned spleen size in cirrhosis is influenced by the underlying aetiology [107]. However, if this finding raises an argument on the accuracy and usefulness of spleen size as a "marker" of PH, on the other

hand it strengthens even more the usefulness of SS which was shown to be independently correlated to PH regardless of splenic size.

Previous studies showed that LSPS is an excellent predictor of CSPH. Nevertheless, it was used in populations composed by HCV-related cirrhosis where probably a linear correlation is found between PH and splenic size. The results of this study reveal that in the presence of a heterogeneous population with different aetiologies SS performs better than LSPS.

Finally the results showed that LS and SS can be used during the same ultrasound examination to distinguish CPH from NCPH and therefore characterise PH. The liver and spleen are coupled organs due to their communication through the portal venous system. This link is particularly obvious in cirrhosis in which the increased intrahepatic resistance reflects through the portal venous system on splenic congestion and the development of collateral vascular circulation. In this clinical scenario LS is typically increased owing to advanced fibrosis and SS is increased accordingly for the above-mentioned reasons reflecting the severity of PH. In NCPH due to EHPVO there is a disconnection between these two organs. Liver parenchyma is typically not affected or marginally affected since it never develops significant fibrosis and stiffness is usually normal or slightly increased unless there are other confounding pathophysiological factors (e.g cholestasis due to choledocical varices, involvement of hepatic veins in overlap Budd Chiari Syndrome, nodular regenerative hyperplasia in patients with haematological disorders). The spleen instead is typically increased in size and extremely stiff (Fig 34). It is of note that the majority

of patients in our population of NCPH were affected by MPN in which the spleen is often involved and this probably reflects also the underlying splenic infiltration. Nevertheless all these patients had CSPH complicated by the development of large varices as shown on CECT; and the two patients with PVT secondary to pylephlebitis had similar features in terms of LS and SS measurements. Platelet count was normal in all patients with NCPH representing a distinctive feature compared to the low platelet count of patients with CSPH due to cirrhosis. It should be highlighted that the population of this substudy does not include cases of IPH in which often splenomegaly is observed, spleen stiffness is high in the presence of CSPH and liver stiffness can be slightly/moderately increased but without reaching the values found in cirrhosis. Platelet count is usually low as a consequence of hypersplenism.

Finally it needs to be remarked that SS might be relatively low in the presence of PH complicated by large vascular shunts which decongest the portal venous system and in the presence of iatrogenic shunts such as TIPSS. It might be slightly increased, but not to the extent seen in the presence of CSPH, in patients with lymphoproliferative disorders or splenomegaly secondary to increased lymphoid hyperplasia which can be observed in patients with PSC and underlying Inflammatory bowel disease.

In summary our study shows that liver and spleen stiffness measured by ElastPQ is an excellent method to assess liver disease. It provides rapid and accurate information on the amount of fibrosis and severity of PH which could be particularly useful before endoscopic screening for oesophageal varices or in the pre-assessment for patients who are in the need to

undergo liver resection for HCC. Particularly regarding variceal screening, although Baveno Consensus recently provided general guidence on the cutoff values for predicting the presence/absence of oesophageal varices, the aetiology of liver disease evaluated regarding those values was mainly virus-related. Although the sample population of this study is relatively small, it was shown that SS has the potential to provide better prognostic information compared to LS since it is more influenced by extrahepatic factors, which are involved in more advanced stages of liver disease. In addition it is less influenced by aetiology, liver inflammation, cholestasis and is truly a more reliable expression and surrogate marker of PH. Nevertheless it is the overal evaluation of the coupling of liver and spleen that provides the most useful information for the understanding of liver disease and the presence and characterisation of PH.

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