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LIVER AND SPLEEN SHEAR-WAVE ELASTOGRAPHY IN THE DIAGNOSIS AND SEVERITY STAGING OF PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE DISEASES AND MYELOFIBROSIS

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Abstract

Introduction: Spleen and liver stiffness, investigated by transient elastography (TE), have been associated with marrow fibrosis in patients with Ph-negative myeloproliferative neoplasms (MPNs). Tissue stiffness can be assessed by shear wave elastography (SWE), the two most common techniques being point (pSWE) and bidimensional (2DSWE). Aims of this study are: 1) to identify TE differences between MPN pts, cirrhotics and healthy volunteers (HV); 2) to evaluate specific TE features in pts with MF, PV and ET; 3) to establish a correlation with bone marrow fibrosis grade.

Methods: In this monocentric study, MPN and cirrhotic pts and HV received elastometric evaluation of spleen and liver stiffness by pSWE and 2DSWE.

Results: A total of 236 pts were included in this study: MPN pts were affected by MF (64, 27.1%), PV (33, 14%) or ET (46, 19.4%), in addition to 75 (32%) healthy controls and 18 (8%) cirrhotic volunteers. Compared to HV, MF patients had greater spleen (pSWE 40.9 vs 26.3 kPa, $p<0.001$; 2DSWE 34.9 vs 20.1 kPa, $p<0.001$), and liver stiffness (pSWE 7.72 vs 5.52 kPa, $p<0.001$; 2DSWE 6.96 vs 5.01 kPa, $p<0.001$). Compared to PV and ET pts, MF pts had higher spleen ($p<0.001$) and liver stiffness ($p<0.001$). In low (0-1) (n=81 , 60.4%) versus high grade fibrosis (2-3) (n=42, 39.6%), is evident a higher stiffness in patients with higher grades of bone marrow fibrosis both for liver (pSWE 5.2 vs 6.65 kPa; 2DSWE 5.1 vs 6.05 kPa) and spleen (pSWE 27.2 vs 37.9 kPa, 2DSWE 21.7 vs 30.75 kPa – $p<0.001$ in both tests).

Conclusions: TE evaluation distinguishes MF pts from HV and ET/PV. TE data were significantly associated with prognostically relevant features including marrow fibrosis in all MPNs. Overall, spleen/liver stiffness may help in MPN diagnosis and may provide clinical guidance.

Part 1 - Background

Myeloproliferative disorders

Chronic myeloproliferative neoplasms (MPNs) are classified into eight different disorders: polycythemia vera (PV), primary myelofibrosis (PMF), essential thrombocytosis (ET), chronic myeloid leukemia (CML), bcr-abl positive chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL), mastocytosis and other unclassifiable myeloproliferative neoplasms. These entities have an origin in a hematopoietic cell, with the subsequent production of one or more of fully formed, non-dysplastic blood cells, myelofibrosis, a tendency for extramedullary hematopoiesis and transformation to acute leukemia. CNL, CML and CEL express a myeloid phenotype, while PV, PMF and ET express a predominantly erythroid phenotype: in the first case due to chromosomal translocations and in the second by driver mutations that activate directly or indirectly JAK2, a tyrosine kinase essential for the activation of erythropoietin and thrombopoietin receptors.

Primary Myelofibrosis

PMF is a condition that has its main hallmark in bone marrow fibrosis: this is originated by a clonal hematopoietic myeloproliferation with an associated megakaryocytic hyperplasia, that provoke a fibroblastic proliferation and deposition of excess collagen, stimulated by the mediation of TGF-beta.

This causes a clinical phenotype consisting of reduced hematopoiesis and, consequently, anemia. Notably, this causes extramedullary hematopoiesis, with foci in different tissues (the spleen and the liver first, but also skin, nervous system, lungs, retroperitoneum).[1], [2] Etiology is unknown, and there are no definite causes for PMF: a somatic mutation of a hematopoietic cell is thought to cause this condition.[3] Anomalies at the cytogenetic level are chromosomal, most often a deletion of the segment of the segment with RB gene, or a partial trisomy of 1q. There are many pathogenetic mutations, the most prominent being JAK2, and secondarily MPL and CALR, some of them are

associated with different disease phenotypes or survival expectancy[4], [5]. JAK2/STAT constitutional activation has been observed in 50% of PMF patients, CALR in 35% and MPL around 5% of patients [1]. PMF is the least represented of the MPNs, and typically affects older adults.

Clinically, the main symptom is fatigue, in more than 50% of the patients, then abdominal discomfort due to a grossly abnormal splenomegaly, almost invariably present in every patient with PMF, which is usually the trigger for the diagnosis[6], [7]. Enlargement of the spleen and the liver are typical, and caused to the extramedullary hematopoiesis, especially the first one is the hallmark of PMF, with massive splenic diameter, often stretching in the lower left abdominal quadrant and in the epigastrium: early gastric repletion, a heavy sensation in the left upper quadrant, or pain due to splenic infarction are common symptoms[8]. Hepatomegaly is also common and sometimes associated with portal hypertension due to increased portal vein flow. The osteoarticular system is also involved: increased bone density, secondary gout due to increased uric acid and turnover of blood cells are the most frequent findings. Blood tests in these patients often show anemia with hemoglobin level less than 10 g/dL, due to the reduction of erythropoiesis in the bone marrow, the ineffective erythropoiesis in extramedullary foci and the increased splenic sequestration due to splenomegaly[9]. Variable counts of platelet and white blood cells are reported in PMF, both over and under the normal limits. Thrombocytopenia is typically associated with a more progressed disease, in parallel with abnormal platelet function[10]. Other abnormal tests are elevated ALP due to liver and bone hematopoiesis, LDH and uric acid, for the increased blood cell turnover[11].

However, diagnosis relies mainly on bone marrow biopsy (the bone marrow aspiration is not sufficient, as it yields often a “dry” tap), that demonstrates extensive fibrosis more visible with the reticulin stain[12]. Diagnosis is made according to the diagnostic criteria showed in Table 1 for Overt PMF, and in table 2 for pre-PMF.

Overt PMF – diagnostic criteria
Major criteria
1. Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3
2. Not meeting WHO criteria for ET, PV, *BCR-ABL1*$^+$ CML, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or, in the absence of these mutations, presence of another clonal marker, or absence of reactive myelofibrosis
Minor criteria
Presence of at least 1 of the following, confirmed in 2 consecutive determinations:
a. Anemia not attributed to a comorbid condition
b. Leukocytosis $\geq 11 \times 10^9$/L
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range
e. Leukoerythroblastosis
Diagnosis of overt PMF requires meeting all 3 major criteria, and at least 1 minor criterion

Table 1:from Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016

Pre-PMF – diagnostic criteria
Major criteria
1. Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted bone marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis
2. Not meeting the WHO criteria for *BCR-ABL1*$^+$ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker,* or absence of minor reactive bone marrow reticulin fibrosis¶
Minor criteria

Presence of at least 1 of the following, confirmed in 2 consecutive determinations:
a. Anemia not attributed to a comorbid condition
b. Leukocytosis ≥11 × 10^9/L
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range
Diagnosis of prePMF requires meeting all 3 major criteria, and at least 1 minor criterion

Table 2: from Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016

Differential diagnosis has to take account of other clinical entities such as acute myelofibrosis, a rare acute myeloid leukemia with an acute onset of bone marrow fibrosis, to be promptly recognized and treated with chemotherapy and hematopoietic cell transplantation[13]. Secondary myelofibrosis is instead present in patients with an history of another MPNs that develop a bone marrow fibrosis, accompanied by worsening of anemia, splenomegaly, and development of constitutional symptoms[14].

The objective in PMF is to determine the patient's prognosis according to the different risk profile of the patient and then deciding which kind of therapy is optimal. Dynamic international prognostic scoring system (DIPSS) is scoring system that classifies patients according to risk classes (from low, to intermediate-1 and -2 to high-risk) according to many clinical features (age, leukocyte count, hemoglobin, circulating blasts, constitutional symptoms)[15]. In high-risk patients, allogenic hematopoietic cell transplantation is preferred[16], while in lower risk classes patients are usually enrolled in clinical trials. If patients are not amenable for enrollment in these trials, symptoms relief is usually obtained with different molecules: kinase inhibitors such as ruxolitinib and pacritinib or hydroxyurea are the licensed ones. Ruxolitinib, in particular, is an inhibitor of JAK2 with an efficacy proven in JAK2-negative patients, especially in symptoms relief such as splenic volume reduction, but does not prolong survival in PMF patients[17]. Prognosis is extremely variable and depends on the risk class and the therapy received by the patient.

Polycythemia Vera

PV is a hematopoietic stem cell disorder, characterized by an elevated red cell mass without a definite physiologic stimulus. A mutation in the inhibitory domain replacing valine with phenylalanine (V617F) tyrosine kinase JAK2 causes its constitutional activation by autophosphorylation, a feature expressed by 95% of PV patients. Other mutations on genes such as calreticulin (CALR) and LNK, a JAK2 inhibitor, cause a similar phenotype.

Clinically, PV is often diagnosed after the incidental finding of an increased hematocrit, hemoglobin or red cell count. In parallel, isolated thrombocytosis, and splenomegaly are often found. Erythrocytosis causes increased blood hyperviscosity, with associated neurologic symptoms, the most serious one being transient ischemic attacks. Erythromelalgia is another associated symptoms complex consisting of burning, painful and erythematous limbs[18]. Budd-Chiari syndrome, an obstruction of the hepatic veins, is rather common in association, and PV should be actively investigated when Budd-Chiari is diagnosed.

Cornerstone of the diagnosis is erythrocytosis, especially when paired with thrombocytosis, leukocytosis, and splenomegaly. When only the first sign is present, or hemoglobin level is ≤20 g/dL diagnosis is more complex and needs to be distinguished by secondary causes of erythrocytosis[18]. A genetic assay to look for a JAK2 mutation is mandatory in these cases, while no specific cytogenetic findings are associated with PV. PV complications are related to the increased viscosity due to the increased number of red cells, such as the increased risk of thrombosis and their increased turnover, causing gout. However, the most important one is secondary myelofibrosis, that is part of the natural history of this condition. In a substantial percentage of patients, MF is associated with hematopoietic stem cell failure, with a parallel extramedullary hematopoiesis in sites such as spleen and liver, causing massive splenomegaly and subsequent portal hypertension other than mechanical discomfort. Acute leukemia is rare. Pruritus can be present due to the activation of mast cells by JAK2[19], pain

due to splenic infarction, and peptic ulcer disease is frequently associated with PV due to an increased association of H. Pylori infection[20].

Treatment has always had its bases in phlebotomy[21], to reduce hemoglobin levels under 14 g/dL in men and 12 g/dL in women, and in aspirin to prevent thrombosis[22], although evidence is scarce: anticoagulation is reserved to patients who experienced thrombotic events. Drugs such as ruxolitinib, pegylated interferon-α and hydroxyurea are in use in clinical practice, with different roles. Ruxolitinib, a JAK2 inhibitor, has been proved to reduce the spleen size and the patient' symptoms burden[23]; PEG-IFN-α induces a complete remission, both hematologic and molecular in 1/5 of the patients[24]; hydroxyurea is a cytotoxic drug that reduces the rate of thrombotic events [25]. Nevertheless, PV is an indolent disease, and can be managed mainly with phlebotomy.

Essential Thrombocythemia

ET manifests itself clinically with an increased platelet count, caused by a clonal proliferation of myeloid progenitors, and is associated with mutations such as JAK2, MPL and CALR: the vast majority of the cases are sporadic, although familiar cases are reported in the literature[26]. These mutations have different pathogenetic pattern for ET: overproduction of thrombopoietin (TPO), an increased sensitivity of progenitor cells to TPO or the formation of a megakaryocyte colony. This condition has a higher incidence in black people and in female rather than male[27]. Diagnosis is often incidental, with the finding of thrombocytosis in a complete blood count. Again, the most frequent symptom is abdominal discomfort due to splenomegaly, but many more due to microvascular disturbances have been reported, such as headache and transient visual disturbances, syncope, atypical chest pain. The consequence of an increased platelet count, due to changes in platelet aggregation, other than the sheer amount is a higher risk of thrombosis and hemorrhage[28]. Thrombotic events reported are stroke, transient ischemic attacks, retinal artery ischemia, pulmonary embolism, portal vein thrombosis and digital ischemia that usually starts with a Raynaud phenomenon [29]. Bleedings are also frequent, especially when associated with an extremely high platelet

count[28]. Of note, women have an increased risk of pregnancy loss, other than preeclampsia, premature delivery, or stillbirth[30]. Lab anomalies are characteristic: marked thrombocytosis in a peripheral blood smear with anisocytosis, with normal white and red blood cells. Dacryocytes, poikilocytosis and nucleated erythrocytes are found in cases evolved to myelofibrosis. Bone marrow shows only mature megakaryocytes with lobulated nuclei. As for genetic mutations, the most frequent is JAK2 (60%), then CALR (20%) and MPL (5%), although triple negative patients have been found in around 15% of ET patients[31]. Diagnosis usually starts after an incidental finding of thrombocytosis, otherwise unexplained at a blood cell count. WHO criteria include requires the fulfillment of these criteria[32]:

Major criteria

- platelet count over 450,000/microL,

- bone marrow biopsy with enlarged, mature megakaryocytes with hyperlobulated nuclei

- WHO criteria for other Philadelphia negative neoplasms not met

- presence of JAK2, CALR, MPL mutation

Minor criteria

- No other cause of thrombocytosis or demonstration of another genetic mutation.

Diagnosis is made with the fulfillment of 4 of the major criteria or three major criteria and the minor criterion. Other diseases that have to be differentiated from ET are secondary thrombocytosis, PV, PMF[32].

Treatment of ET starts from stratifying patients according to risk classes, based on previous history of thrombosis, age and presence of a clonal JAK2 V617F mutation[33]. Drugs such as aspirin (low doses) can help manage symptoms and reduce thrombotic events in low-risk patients[34] while high risk patients usually have to undergo cytoreductive therapy in addition. Hydroxyurea is recommended in patients with a history of venous and arterial thrombosis (plus aspirin or anticoagulation), or older

than 60 years with the presence of a JAK2 V617F mutation. It has been proven to reduce platelet counts and the risk of recurrent thrombosis[35]. Side effects are reported (mucocutaneous toxicity, mainly), usually mild but drug discontinuation is sometimes needed. Hydroxyurea is teratogen and therefore contraindicated in pregnant women. Pegylated interferon controls the platelet count in ET and reduces the risk of thrombosis, and due to its immunomodulatory properties can reduce the clonal megakaryocytic activity, plus, has a favorable toxicity profile compared to hydroxyurea and anagrelide[36]. Follow-up should be based on risk profile and take account of clinical features such as thrombotic and hemorrhagic events, spleen and liver size, platelet count. Transformation to acute leukemia, myelodysplastic syndrome or myelofibrosis are possible, and are index of a poor prognosis. Otherwise, the majority of ET patients have a normal life expectancy.[37]

Liver and spleen elastography - current employment in clinical practice

Elastography has been used for a long time to non-invasively evaluate stiffness of liver tissue. The additive damage caused by different etiologic factors (viral, autoimmune, alcoholic, steatosis) provokes a deposition of fibrosis in the liver that ultimately leads to cirrhosis. Elastography is able to stage the degree of fibrosis in the liver reducing the need of invasive techniques such as biopsy. Two main different techniques are used: strain elastography, that measures qualitatively the deformation of tissues, and shear wave-based techniques, on which we'll focus, that measures quantitatively the speed of ultrasound waves in liver parenchyma. [38]–[40]

Transient elastography (TE) is performed with a dedicated instrument (Fibroscan) that is able to measure the liver stiffness by sending a mechanical push and measuring the velocity of its propagation. The instrument is able to return a value, usually in kilopascal (kPA) that expresses the stiffness of the tissue. Five to ten measurements are performed, and the median value is considered the definitive one for the physician to interpret. Moreover, the instrument generates values such as IQR, that are used in the quality appraisal of the measurement (the ratio IQR/median should always be under 30% in a good measurement). [38]

Shear-wave elastography (SWE) is a technique present on many different ultrasound systems, that has the advantage of obtaining a stiffness value while performing an ultrasound examination, thus avoiding the need for additional equipment, and directly visualizing the examined zone. SWE can be present both as point shear wave elastography (pSWE) and multidimensional SWE (2DSWE and 3D-SWE). The main drawback of this technique is associated with the different ultrasound systems that perform SWE on the market, thus reflecting in a lack of scientific evidence due to the difficult comparison among them.[38]

The most validated use of elastography, and especially TE, is in grading fibrosis due to viral hepatitis, both HBV and HCV related, with a strong correlation with Metavir stages, and in particular discriminating cirrhosis from significant fibrosis. [41], [42]

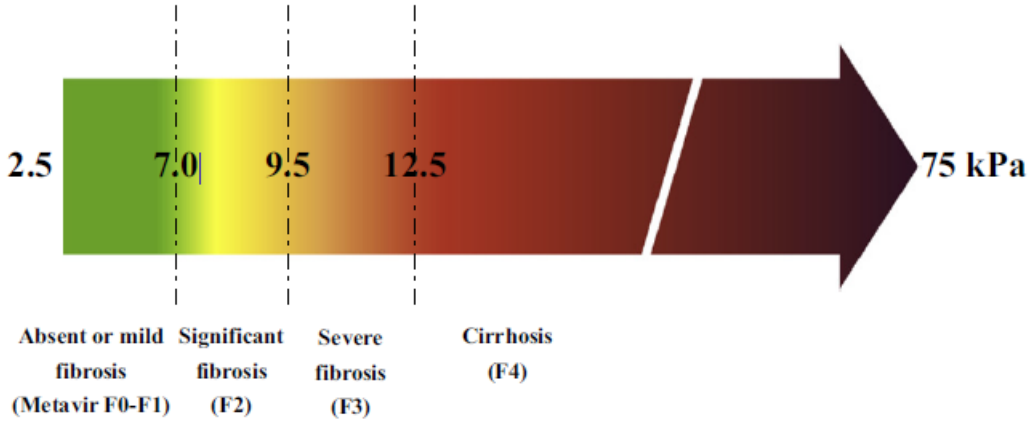


Figure 1: Liver stiffness cutoffs and Metavir correspondance, from Castera et al. 2008

Fewer evidence has been found in the context of non-alcoholic steatohepatitis, in alcohol-related liver disease and in liver autoimmune diseases although it is still used to rule out cirrhosis[43]. Other than that, higher stiffness values have been proven to correlate with portal hypertension, thus predicting cirrhosis decompensation and variceal bleeding[44], [45].

Spleen stiffness is equally used in the context of chronic liver disease, to evaluate both the presence of significant fibrosis and cirrhosis in the liver, and the presence of clinically significant portal hypertension[46], as many studies have proven a correlation between TE, pSWE and 2DSWE values with hepatic vein portal gradient (HVPG), and a good accuracy in predicting the presence of oesophageal varices, especially when paired with liver stiffness, although its role is limited to the rule out and not the varices grading[47], [48].

Liver and spleen stiffness in myeloproliferative diseases – a review of the current literature

The first study to employ TE in patients with MF was the one by Iurlo et al.[49], comprising 88 patients who underwent liver and spleen TE with Fibroscan, showing that both correlated with the severity of bone marrow fibrosis (respectively $p = 0.007$ and $p < 0.001$), and formulated equations to estimate the probability to develop bone marrow fibrosis. Most importantly, this paper showed how liver and spleen stiffness are able to reliably, non-invasively, classify patients in pre-fibrotic and advanced fibrotic stage. Moreover: a small number of patients performed both liver and spleen TE before and after undergoing ruxolitinib therapy, with a substantial variation of spleen stiffness paralleled by an improvement in constitutional symptoms, thus suggesting a role in the assessment of the response to therapy.

Webb et al. compared MF patients with cirrhotic and healthy controls, performing a complete ultrasound scan paired with Fibroscan and 2DSWE (SSI, France) on liver and spleen. Mean spleen stiffness was higher in cirrhotic patients than in MF patients: 41.3 vs 58.5 with Fibroscan and 32.9 and 40.5 (kPa) with SWE, respectively, and considerably lower than healthy controls (13.5 for Fibroscan and 18.1 kPa with 2DSWE). This study was the first to use 2DSWE and to differentiate MF patients from healthy controls, however both cirrhotic and MF patients for their high stiffness values are not able to be distinguished.[50]

The first paper to encompass all of the MPNs was published by Benedetti et al. 70 patients with MPNs consisting of 43 with MF, 17 with PV and 10 with ET underwent a full ultrasound scan with pSWE. Splenic median stiffness was significantly higher in MF (53.96 kPa) and PV (90.1 kPa) patients than in controls (27.5 kPa – $p < 0.0001$ and $p = 0.002$, respectively), but not between MPNs. Spleen stiffness strongly correlated ($p < 0.0001$) with the extension of bone marrow fibrosis even in this larger cohort of patients, as the reduction in stiffness value in five patients (4 with MF, 1 with PV) undergoing ruxolitinib therapy. A major strength of this study was the inclusion of a follow-up period,

that allowed the authors to affirm that patients with spleen stiffness values over 40 kPa were associated with a worse progression-free survival.[51]

Another recent paper by Moia et al. confirmed substantially all the previous literature findings: 63 MPNs patients (22 with MF, 9 with PV and 32 with ET) performed Fibroscan with a dedicated spleen module. Higher stiffness values were higher in MF than in PV and ET (p=0.015). and correlated with higher values of bone marrow fibrosis (p=0.035), lower hemoglobin levels (p=0.014) and leukocytosis (p=0.008). Moreover, spleen stiffness correlated with higher JAK2 variant burden (p=0.02).[52]

Therefore, we decided to study the role of two simple to use and rapidly performed techniques such as pSWE and 2DSWE. We included four different patient cohorts: MF, PV, ET, cirrhotics and paired them with healthy controls, trying to confirm if liver and spleen stiffness were able to differentiate among them (and from cirrhosis, the most validated indication for liver and spleen elastography), and especially their role in predicting bone marrow fibrosis.

Part 2 – Research - Liver and spleen shear-wave elastography in the diagnosis and severity staging of Philadelphia-negative Myeloproliferative diseases and Myelofibrosis

Introduction

Chronic myeloproliferative neoplasms (MPNs) are a group of hematological diseases that include polycythemia vera (PV), essential thrombocythemia (TE) and primary myelofibrosis (PMF) These rare diseases greatly affect bone marrow hematopoiesis and cause extramedullary hematopoiesis. PMF, in particular, has a great impact on prognosis and affected patients have a shortened life expectancy.

Diagnosis is established only by bone marrow biopsy. Nowadays, imaging has exclusively a supporting role in the natural history of these patients. Ultrasound, as widely available it is, is part of the routine follow-up for MPNs, to assess splenomegaly, lymph node involvement and other extramedullary hematopoietic foci.

When paired with US, transient elastography has proven to be a valuable tool, in providing information in patients with liver disease, greatly reducing the need for hepatic biopsy. Spleen stiffness, similarly, has proven useful as a surrogate marker of portal hypertension. As of today, however, no role of any of these techniques is established in the work-up of MPNs.

Previous attempts were made to assess the severity of MPNs diseases with transient elastography. Iurlo et al. successfully associated liver and spleen stiffness values measured with Fibroscan with bone marrow fibrosis in a sample of 88 PMF patients.[49] Other groups have implemented 2D Shear-wave (2DSWE), a technique which can measure stiffness during an ultrasound examination, thus visualizing the measured tissue in the work-up for MF. They concluded that 2DSWE could distinguish PMF patients from healthy controls.[50] Benedetti et al. performed point shear wave in

70 MPN patients, finding that liver and spleen stiffness differed between MF patients and the healthy cohort, but not between MPNs, Moreover, spleen stiffness values had a strong correlation with bone marrow fibrosis grade.[53]

Our aim for this study is to investigate whether liver and spleen stiffness measured with both pSWE and 2DSWE could be instrumental in differentiating MPNs from healthy controls and cirrhotic patients, and if MPNs have significantly different values of LS and SS. Moreover, correlation with bone marrow fibrosis, history of splanchnic thrombosis and other clinical variables were explored.

Patients and methods

This is a monocentric, retrospective study, in which from March 2021 to July 2021 a total of 236 patients were enrolled: 64 with MF, 33 with PV, 46 with TE, in addition to 75 healthy controls and 18 cirrhotic volunteers. Clinical data have been collected according to the patients' participation to projects "RUX-MF" and "PV-NET", and subsequent Ethical Committee approval. All patients gave their written consent to data collection when enrolled.

Patients, after an at least six hour fasting period, underwent a complete abdominal ultrasound scan with an Esaote MyLab 9 (probe C 1-8). Doppler US, pSWE and 2DSWE were performed for each patient by the same operator (V.S.), including a full evaluation of the abdominal organs, reporting all relevant measurements in centimeters and millimeters.

More specifically: anteroposterior diameter for the liver, measured through the right intercostal window. Spleen diameter and cross-sectional area, measured at the hilum through the left intercostal window. (Image 1) Portal vein diameter and flow velocity, sampled in the epigastric window at the crossing with the hepatic artery and splenic vein diameter, sampled in its intrapancreatic portion.

pSWE and 2DSWE were performed both for liver and spleen in their respective intercostal windows on supine patients extending their arm behind their head, to increase the acoustic window with the

patient in a still position, holding the breath for 2-3 seconds (Image 2). At least 5 measurements for the liver and 3 measurements for the spleen were recorded, and the ROI was placed at least 2-2.5 cm from the organ capsule. Values were expressed in kilopascal (kPa) and measurement quality was ensured by reporting IQR/M and for pSWE by excluding low quality measurements indicated by the internal quality tool of the ultrasound equipment.

We collected hematological parameters such as bone marrow fibrosis, driver mutations and percentages of allelic burden (categorizing patients in homozygotic and heterozygotic), Dynamic International Prognostic Scoring System (DIPSS) at the time of visit, history of thrombosis and splanchnic thrombosis. Descriptive statistics are listed in Table 1.

Statistical analysis was performed with Stata/SE 17. Categorical variables were expressed by their frequency and continuous data by mean, median and confidence intervals. Continuous variables were compared in different groups with Kruskal-Wallis rank test and Dunn's pairwise comparison. A Mann-Whitney test was used to compare parenchymal diameters and their respective stiffness. A linear regression with ANOVA test was used to compare liver and spleen pSWE and 2D stiffness values.

Results

Regarding pSWE liver stiffness, MF patients had the highest median stiffness values among all other MPNs (6.65 kPa), whilst cirrhotic patients had the highest among all patients (17.65 kPa). A statistically significant difference was reported among MF and all other cohorts, even healthy patients ($p<0.001$), but not between MPNs. For 2DSWE, results were similar: MF had the highest stiffness values among MPNs (6.1 kPa). Again, values were statistically different among MF and all other cohorts, healthy patients included ($p<0.001$), but not between MPNs. Median liver stiffness values are reported in Table 1.

Regarding pSWE spleen stiffness, the highest median values were found in MF (37.95 kPa) and cirrhotic patients (49.5 kPa). Statistically significant difference was reported for MF between all other MPNs ($p<0.001$) but not for cirrhotics ($p =0.379$). 2DSWE yielded similar results: MF had median values of 31.65 kPa and cirrhotic patients had a median stiffness of 40.85 kPa, without a statistically significant difference between the two groups ($p =0.095$). Median spleen stiffness values are reported in Table 2.

Ratios (liver/spleen) were calculated for both pSWE and 2DSWE to help discriminate between MF (median values: pSWE = 0.18, 2DSWE = 0.20) and cirrhotics (median values: pSWE = 0.41, 2DSWE = 0.47) being significantly different for both techniques ($p<0.001$).

A splenic diameter larger than 12 cm was not associated with an increased stiffness both in MF (pSWE, median values 31.1 vs 38.3 kPa, $p=0.222$) and in cirrhotic (median values 64.9 vs 46.2 kPa, $p=0.339$) patients.

In all MPN patients which underwent bone marrow biopsy (n=123), every technique we performed (pSWE and 2DSWE) both for liver and spleen regrouping differently the classes in low (0-1) (n=81 , 60.4%) versus high grade fibrosis (2-3) (n=42, 39.6%), shows a higher stiffness in patients with higher grades of bone marrow fibrosis both for liver (pSWE 5.2 vs 6.65 kPa; 2DSWE 5.1 vs 6.05 kPa) and spleen (pSWE 27.2 vs 37.9 kPa, 2DSWE 21.7 vs 30.75 kPa), with a strong statistical difference ($p<0.001$ in every test). ROC curves were calculated for both splenic stiffness techniques (Image 3), to find a threshold value which could discriminate low grade fibrosis from high grade. For pSWE, a value of 33.1 kPa had a sensitivity of 61.9%, a specificity of 69.2% and a number of cases correctly classified of 66.7%; AUROC for this curve was 0.702. For 2DSWE, a value of 25.8 kPa had a sensitivity of 61.9%, a specificity of 65.4% and a number of cases correctly classified of 64.2%; AUROC for this curve was 0.696.

Splanchnic organ sizes differed between patients: liver right lobe was larger in MF compared to all the other MPNs and cirrhotics ($p=0.002$), but not between healthy volunteers (HV) and other MPNs.

Splenic diameter and area were significantly different between MF, other MPNs and HV. Organ sizes are summarized in Table 3.

We compared splenic stiffness values in 142 patients with MPNs comparing those who did not have an history of splanchnic thrombosis (133 - 97%) versus the other who did (9 – 3%), with median values of 30.9 kPa vs 56.2 kPa; $p<0.001$).

Patients were divided according to two age classes (≤65 years of age and older): while splenic values were not different between the two groups, liver values were instead significantly different for both techniques (lower vs. higher: pSWE: 5.3 vs 6.2 kPa, p=0.009; 2DSWE: 4.9 vs 5.7 kPa, p=0.001)

Regarding allelic burden of the 181 MPN patients with $JAK2^{V617F}$ mutation, splenic values were higher in patients harboring the mutation in homozygosis (141, 78%) than in heterozygosis (40, 22%) (pSWE: 35.3 vs 28.6 kPa, p=0.01; 2DSWE: 22.7 vs 31.2 kPa, p=0.001). Spleen and liver TE values were not significantly associated with different DIPSS risk class.

Lastly, we confirmed a strong correlation between the values obtained with the two techniques, both pSWE and 2DSWE for liver (mean values 7.28 vs 6.76 kPa, R= 0.83, $p<0.001$) and spleen (mean values 32.6 vs 26,2 kPa, R= 0.78, $p<0.001$)

Discussion

Myeloproliferative diseases such as PV, ET and MF are entities that greatly affect patient prognosis. The need for tools to better stratify patients in risk classes is only partially met by a system such as DIPSS. However, such scores do not take into account many clinical parameters of great importance for these diseases. Bone marrow biopsy is still the gold standard to establish a diagnosis and grade the disease severity.

In this study we investigated the role of elastography in differentiating the three MPNs from one another and from healthy controls. To lay a context in which SWE is well-established in the work-up

of cirrhosis, both in liver and in spleen stiffness, we compared a cohort of them with our MPN patients.

Both the techniques we employed, pSWE and 2DSWE, in liver and spleen were able to distinguish all MPNs and especially MF from healthy patients, and MF from other MPNs confirming the findings of the existing literature. Values were significantly different even when compared with cirrhotic patients, except with spleen stiffness, as cirrhotic patients have stiffer spleen due to portal hypertension[54], [55]. However, simply establishing a ratio between the liver and spleen stiffness is capable to differentiate the two cohort of patients, aside from the many other clinical and ultrasound signs used in clinical practice.

Another important finding is the strong correlation of both techniques with the grade of bone marrow fibrosis. Given the irreplaceable role of biopsy in the grading of MPNs, this non-invasive test is capable to supply the physician with a tool capable of signaling the presence or absence of severe marrow fibrosis. We established thresholds for severe fibrosis for pSWE with a value of 33.1 kPa and for 2DSWE a value of 25.8 kPa, with suboptimal values of sensitivity and specificity. Larger cohorts of patients are required to find the right threshold.

Another interesting correlation of splenic stiffness is the one with the history of splanchnic thrombosis, a novel finding that could have important prognostic consequences, although with the bias of a small patient population. Moreover, we confirm the previous findings [52] of the association of allelic disease burden, bearer of a more aggressive disease, with spleen stiffness. As an ancillary finding, older patients do not present themselves with higher values of splenic stiffness, therefore higher values depend exclusively on the disease.

Among the strengths of this study there is the use of SWE, that allows direct visualization of the splenic parenchyma, while, in the same instance, performing the ultrasound exam with the same machine. Healthy controls and cirrhotic patients were included as cohort to provide a better context

for stiffness values. The main drawback consists of the absence of a follow-up period, to better suffragate our prognostic data.

In conclusion, we successfully showed how liver and spleen shear wave elastography were able to correctly discriminate MF from other MPNs and healthy controls. Moreover, we were able to confirm a strong association of spleen stiffness with the grade of bone marrow fibrosis, strengthening the role of elastography as a viable non-invasive surrogate parameter.

Tables and figures

Table 1 – Descriptive statistics of the patient population

Characteristics	MF (n.63)	PV (n.33)	ET (n.46)
Median age, yrs (range)	71.9 (44.6-83.4)	62.4 (27.5-91)	64.4 (20.8-89.4)
Male sex, n. (%)	38 (60.3%)	22 (66%)	19 (41%)
Leukocyte (x10^9/L)	8.2 (1.8-60.2)	8.3 (2.1-18.3)	7.1 (3.4-15.6)
Hemoglobin (g/dl)	10.4 (6.7-16.9)	13.9 (10.9-16.6)	13.5 (9.4-16.9)
Platelet (x10^9/L)	210 (18-779)	386 (138-873)	509 (107-1800)
Previous Thrombosis, no. (%)	13 (20.6%)	8 (24.2%)	10 (21.7%)
Previous Splanchnic Vein Thrombosis, no. (%)	6 (9.5%)	2 (6%)	1 (2.1%)
Marrow fibrosis, no. (% on evaluable) **grade 0** **grade 1** **grade 2** **grade 3**	 1/56 (1.8%) 14/56 (25.0%) 19/56 (33.9%) 22/56 (39.3%)	 12/17 (70.6%) 5/17 (29.4%) 0 0	 23/34 (67.7%) 11/34 (32.3%) 0 0
Ongoing therapy, no (%) **Cytoreduction** **Interferons** **JAK2-inhibitors** **No therapy**	 10 (15.9%) 1 (1.6%) 33 (52.4%) 19 (30.2%)	 20 (60.6%) 5 (15.2%) 2 (6.1%) 6 (18.2%)	 36 (78.3%) 2 (4.3%) 0 8 (17.4%)
Median total symptom score	13 (0-48)	5 (0-39)	-
DIPSS, no (%) **low** **intermidiate-1** **intermidiate-2** **high**	 5 (7.9%) 32 (50.8%) 19 (30.2%) 7 (11.1%)	-	-
Palpable spleen, cm BLCM	5 (0-28)	0 (0-8)	0
≥1 HMR mutations, no	11/23	-	-
Median time from diagnosis, mos	3.7 (1-235.3)	4.1 (1.5-375.1)	6.6 (1-260.7)
Median PVD (mm)	12 (5.7-21.4)	10.7 (6.6-19)	9.4 (7.1-15.6)
Median SVD (mm)	9.2 (4.7-22.7)	7.3 (4.2-20)	7.2 (4-11)

Table 2 - Mean and median values for liver stiffness values in different patient cohorts

		LIVERPOINTSWE (kPa)					LIVER2DSWE (kPa)			
		N	MEAN	MEDIAN	95% CI MEDIAN LOWER	95% CI MEDIAN UPPER	MEAN	MEDIAN	95% CI MEDIAN LOWER	95% CI MEDIAN UPPER
Aetiology	MF	64	7,72±,42	6,65	6,40	7,00	6,96±,32	6,10	5,60	6,80
	HV	75	5,52±,35	5,00	4,50	5,90	5,01±,22	4,62	4,30	5,00
	PV	33	5,73±,35	5,40	4,90	6,20	5,32±,31	5,00	4,90	6,00
	TE	46	5,44±,25	4,85	4,70	5,70	5,23±,23	4,85	4,40	5,30
	CIR	18	20,57±2,38	17,65	14,00	26,20	19,94±3,04	15,05	11,80	24,20

Table 3 – Mean and median values for splenic stiffness values in different patient cohorts

		SPLEENPOINTSWE (kPa)					SPLEEN2DSWE (kPa)			
		N	MEAN	MEDIAN	95% CI MEDIAN LOWER	95% CI MEDIAN UPPER	MEAN	MEDIAN	95% CI MEDIAN LOWER	95% CI MEDIAN UPPER
Aetiology	MF	64	40,9±2,1	38,0	41,4	33,1	34,9±2,1	31,7	26,2	34,3
	HV	75	26,3±1,4	23,4	25,2	21,4	20,1±,7	18,7	17,6	21,1
	PV	33	31,5±2,1	31,1	33,1	26,0	24,6±1,5	22,3	19,7	26,5
	TE	46	26,7±1,6	26,4	29,3	21,7	22,8±1,2	21,1	19,2	25,2
	CIR	18	45,6±4,8	49,5	63,0	35,2	39,4±3,0	40,9	33,4	45,7

Table 4 – Splanchnic organ dimensions

			LIVERRIGHTLOBE (mm)				SPLEENDIAMETER (cm)				SPLEEN AREA (cmq)			
			MEAN	MEDIAN	95% CI MEDIAN LOWER	95% CI MEDIAN UPPER	MEAN	MEDIAN	95% CI MEDIAN LOWER	95% CI MEDIAN UPPER	MEAN	MEDIAN	95% CI MEDIAN LOWER	95% CI MEDIAN UPPER
Aetiology	MF	64	167±3	166	162	173	19,0±,6	18,2	16,6	19,9	141,15±9,78	122,60	98,00	145,50
	HV	75	150±2	150	146	155	10,8±,2	10,6	10,3	11,1	40,23±1,81	37,50	35,00	40,30
	PV	33	154±4	153	148	167	14,4±,5	13,8	13,2	16,3	77,15±5,75	67,70	63,60	92,90
	TE	46	150±2	149	146	155	11,9±,4	11,4	10,2	13,1	52,94±4,27	41,60	36,90	58,30
	CIR	18	152±5	150	142	157	13,9±,7	14,5	12,1	15,3	66,52±6,19	67,10	51,80	86,20

Image 1 – Liver and splenic dimensional assessment

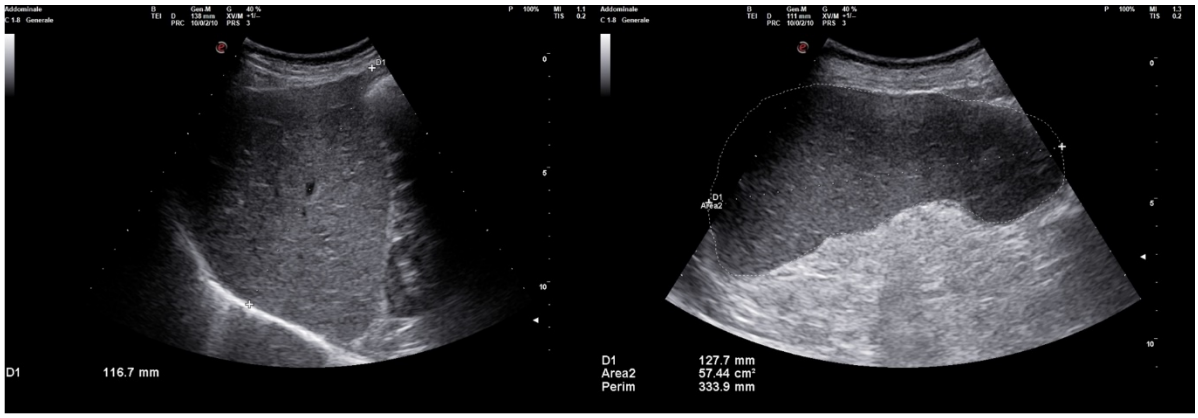


Image 2 – Liver and spleen elastometry, pSWE and 2DSWE

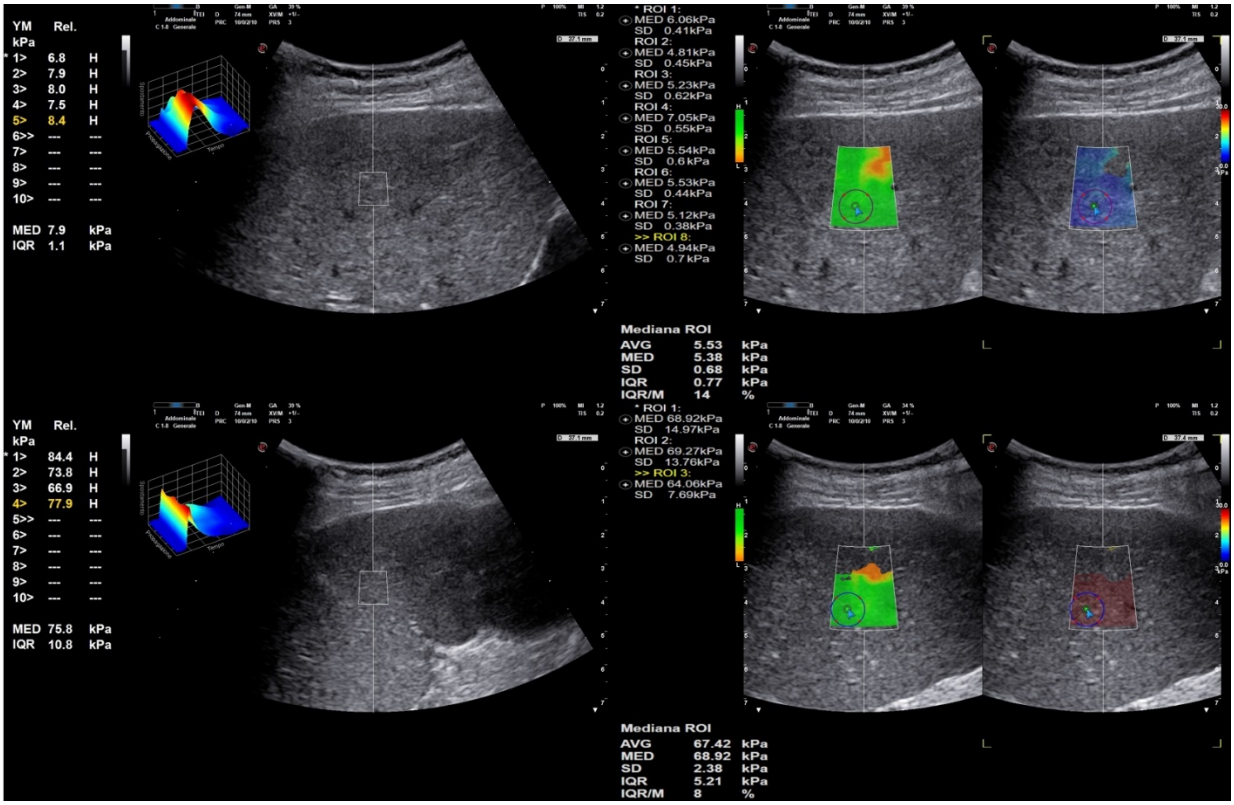
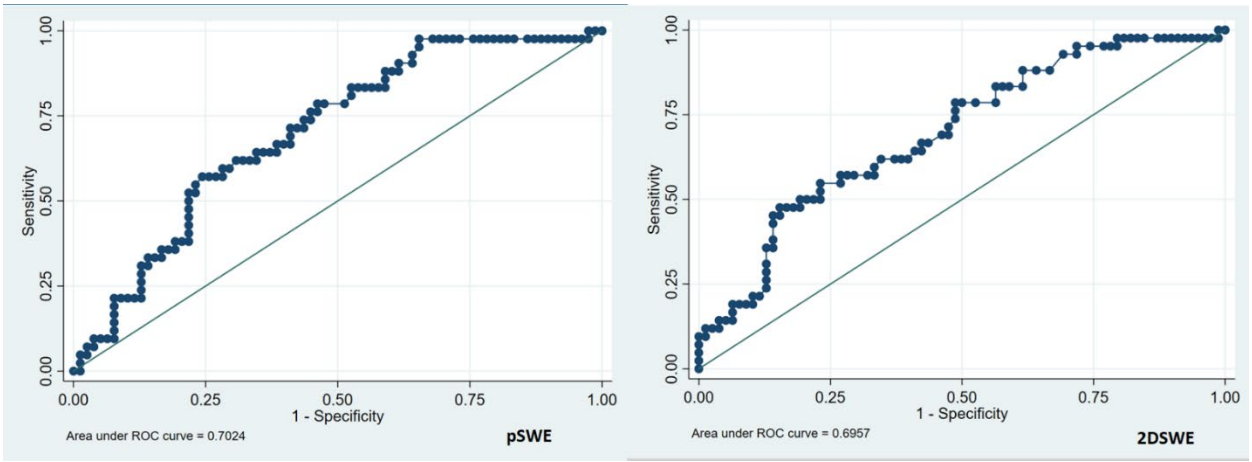


Image 3 – ROC curves for pSWE an 2DSWE spleen elastometry – Bone marrow fibrosis



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