Noninvasive diagnosis of clinically significant portal hypertension in cirrhosis secondary to steatosis

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1. INTRODUCTION

Until recently, screening for clinically significant portal hypertension and high-risk esophageal varices was based on invasive endoscopic procedures. The 2015 Baveno VI Consensus on Portal Hypertension proposed, for the first time, noninvasive biomarkers (platelets and liver stiffness) to better target patients requiring endoscopic screening[18]. This combination has a high sensitivity but low specificity for EV and is extracted mainly from studies where the viral etiology of cirrhosis predominates. We expect this combination of biomarkers to work less well in cirrhosis associated with steatosis. The aim of this study is to evaluate clinical and biological biomarkers that can be correlated with high-risk esophageal varices in cirrhotic patients of non-viral etiology.

2. CURRENT LEVEL OF KNOWLEDGE

2.1. Liver cirrhosis: epidemiology, etiology, clinical picture

Chronic liver disease and cirrhosis are pathologies with high morbidity and mortality, responsible for 1.95% of all deaths globally[1]. Their incidence is decreasing due to the appearance of effective therapeutic options against hepatotropic viruses but increasing due to metabolic (nonalcoholic steatohepatitis) and toxic (alcohol) conditions [2-5].

The etiology of cirrhosis is related to the etiology of the underlying liver disease. In most cases, it is multifactorial with some genetic predisposition [6] and an association of several etiological factors that modify the speed of evolution of chronic liver disease to the stage of cirrhosis. Also, the pathological aspects differ according to etiology.

The clinical picture is usually insidious, in the early stages being frequently asymptomatic. The liver is a complex organ with multiple functions,
including metabolism of carbohydrates, fats, proteins and drugs, synthesis functions, storage functions, digestive functions, excretory functions, and immunological functions [7]. In the context of progression, there may be manifestations of organ failure with deficits in hepato-cellular synthesis but also signs associated with bile elimination disorders and secondary to increased pressure in the port system. The systemic clinical presentation is also terrain (age, comorbidities, genetic polymorphism) and etiology dependent.

2.2 From cirrhosis to portal hypertension: pathophysiology, hemodynamics, diagnosis

Liver disease pathogenic mechanisms encompasses a complex pattern of cascading changes only partially understood. Depending on the etiology, the mechanisms of fibrinogenesis may differ. Non-alcoholic steatohepatitis (NASH) involves not only steatosis, but also inflammation, which in some patients is associated with progressive fibrosis, cirrhosis, and occasionally hepatocellular carcinoma. This process appears to be caused by insulin resistance-induced lipotoxicity and cellular metabolism dysfunctions that eventually progresses to chronic apoptosis, fibrosis, and cirrhosis. From hepatocytes and immune cells to adipose tissue and endothelium, several actors are involved in the evolution of NASH. Their action is modulated by genetic predisposition and environmental factors such as diet and intestinal microbiota. In alcoholic steatohepatitis, the trigger is alcohol toxicity. Direct alcohol toxicity leads to dysfunction of hepatic intracellular lipid metabolism. The fibrosis pathways has some common elements with non-alcoholic steatohepatitis: apoptosis is a central mechanism, driven by the activation of macrophages and fibrosis is mediated by stellate cell activation.

These pathways differ from those found in viral hepatitis. In chronic C virus hepatitis, the innate immune response is the main line of defense, with a role in limiting viral replication[8]. Dendritic cells, NK and NKT nonspecifically attack infected cells. Autophagy, another mechanism triggered in the context of viral C infection, is stimulated directly by the virus and by stress in the endoplasmic
The adaptive immune response occurs in a second phase by attracting B and T lymphocytes following the migration of activated dendritic cells into lymphoid tissues. In chronic B virus hepatitis adaptive immunity activation is responsible both for the pathogenesis of chronic hepatopathy and for viral clearance [10, 11]. Cytotoxic T lymphocytes are responsible for severe hepatic necro-inflammatory reaction after exposure to HBS antigen. Persistence of infection occurs in the case of a defective immune response[12]. Although the immune pathways are different, chronic B or C virus infections lead to destruction of hepatocytes with architectural changes and cirrhosis.

Regardless of the etiology of cirrhosis, the increase in portal pressure occurs in parallel with the evolution of fibrosis[13]. Portal hypertension is a dynamic model of interaction between the aggressor, various host cells, mediators, and the terrain. It involves several corroborated mechanisms. A purely mechanical mechanism results from the modification of the hepatic architecture is associated with an adaptive mechanism at the level of tone of the vascular unit, an angiogenetic mechanism and last but not least, one tied to the formation of porto-systemic shunts.

From a hemodynamic point of view, cirrhosis is associated with local but also systemic changes secondary to the interaction of mechanical, cellular and humoral factors. The interactions between them are complex and only partially known (Figure 1).

The diagnosis of portal hypertension remains mainly invasive, by direct measurement of the portosystemic pressure gradient (HVPG) or by endoscopy. At HVPG values below 10 mmHg, clinical manifestations are generally absent. At values above 10 mmHg it predicts the appearance of esophageal varices and at values above 20 mmHg it predicts variceal bleeding and mortality[14]. Clinically relevant portal hypertension corresponds to the existence esophageal varices at risk of bleeding. Early and accurate diagnosis and staging of liver disease and portal hypertension are crucial [15] because therapeutic interventions have led to a decrease in mortality from over 30% in the 1990s to around 10% in 2015[16] but only when the appropriate therapy is performed in a window of opportunity.
2.3 Non-invasive diagnosis of portal hypertension - the utility of non-invasive biomarkers

Invasive diagnosis is burdened by several limiting factors such as access to technical means, tolerance, and complications. Direct measurement of gate pressure or HPVG are reserved mainly to academic centers and their applicability in real life is limited. Endoscopy is resource consuming and remains an invasive screening test with mediocre tolerance. To facilitate the follow-up process, a series of non-invasive biomarkers associated with the existence of clinically significant portal hypertension are proposed.

Biomarkers are objective, quantifiable characteristics of biological processes[17]. The latest recommendations of the Baveno Consensus (VI) took the first step towards a non-invasive follow-up of portal hypertension by introducing 2 biomarkers, respectively the number of platelets and the stiffness of the parenchyma measured with transient elastometry[18]. Their usefulness is partially
evaluated. In addition, other biomarkers can be used in screening and follow-up. Clinical biomarkers have not been evaluated in the context of portal hypertension. Among the biological biomarkers, some showed a correlation with the existence of clinically manifest portal hypertension - thrombopenia and prothrombin time, and others are evaluated mainly in the context of fibrosis marker - hyaluronic acid, alpha2 macroglobulin or haptoglobin. BNP may be interesting as a biomarker of portal hypertension given the close link between the occurrence of hyperkinetic cardiomyopathy and portal hypertension, and markers of inflammation such as procalcitonin or CRP in the context of bacterial translocation present. Imaging biomarkers can provide morphological information (the size of the liver, spleen or vessels) and doppler techniques provide dynamic blood flow information. Both techniques have already been evaluated in predicting the existence of portal hypertension. Elastometry - by the transient method (FibroScan) - is relatively well documented in the evaluation of portal hypertension from cirrhosis of viral etiology.

Considering the limits of the ability of a single biomarker to predict the existence of clinically manifest portal hypertension and its somewhat linear evolution with fibrosis, well-known predictive scores of fibrosis have also already been tested in noninvasive diagnosis of clinically significant portal hypertension. Although included in international recommendations, none of these biomarkers really consider the etiology and terrain of a cirrhotic patient.

2.4 From biomarkers to scores and back to personalized medicine

The roles of biomarkers are multiple: they can help to understand the disease or the risk of disease, they can aid clinical decisions or motivate the patient to make behavioral changes that improve health. Cirrhosis is a heterogeneous disease, depending on the etiology and mechanisms involved in its occurrence and one should consider that biomarkers will differ between patients. Depending on each person's priorities, social and economic background, their perception and
current knowledge of the disease, biomarkers that fall into a certain risk category can help decide tailored follow-up and treatment.

2. PERSONAL CONTRIBUTION

3.1 General objectives

The main objective of the study is to identify non-invasive clinical, biological or ultrasound biomarkers able to predict the existence of esophageal varices at risk of bleeding in steatosis induced cirrhotic patients.

3.2 General research methodology

It is a "real life" study of patients with non-viral cirrhosis. It is a cross-sectional, observational, monocentric cohort study (Charleville Regional Hospital, France). All patients with suspected cirrhosis who underwent gastroscopy were prospectively included for 8 months in late 2016 and early 2017. The study protocol was accepted by the ethics committee of the institution (June 2016), and the explorations were considered part of standard clinical practice (articles L.1121-1 paragraph 1 and R1121-2, French Public Health Code). Informed patient consent was documented. The data were analyzed in anonymous format.

Study population: Patients with confirmed liver disease and recent gastrointestinal endoscopy without associated pathologies from the list of exclusion criteria.

Inclusion criteria: adults (18-75 years old) with a confirmed liver disease of cirrhosis type on at least 2 concordance tests (APRI and Fib4), who express their written consent to participate and have performed one with recent endoscopy.

Exclusion criteria: Uncontrolled extra-hepatic disease or with limited prognosis (6 months), chronic renal failure stage IV-V; NYHA heart failure III-IV; History of endoscopic band ligation.
Data collection and variables

*Upper GI Endoscopy* - Endoscopic examination of the esophagus, stomach and duodenum was performed using an Exera III (Olympus TM) device in accordance with the guide "Société nationale de gastroenterologie (SNFGE)" by any of the four local gastroenterologists and suggestive features (photos) have been documented. Esophageal varices were classified as absent, small, medium, or large and red marks were marked according to international standards. It refers to "high-risk esophageal varices" in the presence of medium or large esophageal varices or small esophageal varices with red marks.

*Biomarkers* - The following documented biological biomarkers were: hemoglobin, platelets, albumin, prothrombin time, total bilirubin, TGO and TGP, GGT, alkaline phosphatase, creatinine, total cholesterol, C-reactive protein, Procalcitonin, lactate acid, BNP, hyaluronic acid, alpha-2-macroglobulin, haptoglobin, apolipoprotein A2.

The following variables were considered as potential confounders: demographics, comorbidity, treatment at inclusion, alcohol consumption, previous medical history, vital and clinical signs, suspected infections and site, laboratory data. Data on each of these factors were collected from the patient's medical record. The compound scores used were: APRI, Hepascore, FIB4, MELD, LOK, Child Pugh, Charlson comorbidity index.

*Imaging studies* (ultrasound, computer tomography or abdominal MRI) were performed on all patients to document the size of the liver and portal vein, the size of the spleen with bipolar diameter and any signs of portal hypertension.

*Liver stiffness* was assessed either in-hospital or outpatient, by transient elastography (TE), with a standard sample by an experimental operator. (Fibroscan, Echosens, model 402).

To ensure the objectivity of the information, the professionals performing all laboratory tests and imaging techniques did not have at their disposal the clinical and endoscopic data of the subjects.
Ethical considerations

This study is considered to evaluate current practices, patients benefiting from a follow-up that falls within the current recommendations of specialized societies. The patient's informed consent was obtained. The study was approved by the ethics committee of the center (Charleville-Meizeres Hospital). Confidential data will be treated according to the legal provisions in force. It is estimated that the benefit-risk balance is positive for the patient, without any problems specifically induced by the measurements required in the study.

Statistical analysis

Statistical analysis was performed using SPSS v22.0 (IBM, Chicago, Illinois), and a p value <.05 was considered significant. Binary variables were evaluated according to frequencies. Descriptive data with normal distribution (skewness> 0.5) are presented as Mean / Standard Deviation, data with non-normal distribution are presented as Median / IQR. Missing values were replaced with the variable mean.

Statistical differences between groups were analyzed using the Mann-Whitney U test, the Fisher or Chi-square test depending on the type of distribution. The correlation with the main criterion was evaluated using Spearman's or Pearson or correlation coefficients. The area under the curve (AUC) for specificity and sensitivity was calculated. Cut-off values were calculated for maximum sensitivity, maximum specificity, and maximum sensitivity / specificity for variables significantly associated with the existence of esophageal varices at risk of bleeding. Positive and negative predictive values were extracted. Following a binary logistic regression, a model based on these cut-offs was developed, including variables with statistical discriminative capacity between the two groups.
3.3 Results

The study population consists of 50 patients, of which 41 were included in the final analysis. They have an average age of 60 years and are 26 men and 15 women. Among the patients excluded from the analysis, one patient had an exclusion criterion (newly diagnosed neoplasia) and one patient withdrew his consent to participate in the study. 7 patients had chronic, pre-cirrhotic liver disease. None of the 7 had esophageal varices. The etiology of cirrhosis in the studied population is mainly alcoholic in 25 patients, isolated NASH in 6 patients and 10 ten patients had a mixed origin (alcohol and NASH). Ascites is present in 24 patients, 6 patients associate different degrees of clinically manifest encephalopathy, the average albumin is at 29 g/dl, the average bilirubin at 20mg/dl, the prothrombin time at 66% and the average platelet value is 150,000/mm³. The patients average CHILD score is 8 and the MELD score is 10.

Endoscopically, esophageal varices were found in 25 patients and were grade 1 to 6 patients, grade 2 to 13 patients and grade 3 to 6 patients. No ectopic varices were discovered.

Markers with discriminatory potential in portal hypertension, in the population with varices at risk of bleeding versus the population at low risk, are presented in Table 1. Hyaluronic acid, TP and Spleen size are significantly associated with the existence of esophageal varices. Hepascore and FORNS liver fibrosis scores also showed significant differences between the two groups. The detailed values are presented in Table 2. Due to the unjustified absence of more than 20% of the values, the variable Haptoglobin could not be used. Elastometry was performed in 29 of 41 patients, of which 22 (53%) had valid measurements, 7 (17%) patients had failures due to obesity, edema, or ascites.
Correlation between non-invasive markers and high-risk esophageal varices

For biomarkers significantly associated with portal hypertension, the curve under the curve was analyzed for the sensitivity and specificity of the diagnosis of large esophageal varices, presented in Table 4 and Figure 2 compared to the Baveno VI criteria (Table 3).

![ROC Curve](image)

**Table 1.** Esophageal varices with high risk of bleeding versus low risk of bleeding. Results are presented as median / IQR. *p* * statistically significant: Mann-Whitney U unless otherwise recommended (biomarkers with normal distribution are presented mean / SD, *p* * statistically significant: t-test (#)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>High risk varices (N=19)</th>
<th>Low risk varices (N=22)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count (10^9/mmc)</td>
<td>87.5</td>
<td>60</td>
<td>119</td>
</tr>
<tr>
<td>Creatinine (mcmol/l)</td>
<td>70</td>
<td>39</td>
<td>68.5</td>
</tr>
<tr>
<td>ALT (UI/l)</td>
<td>25.5</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>Gamma-GT (UI/l)</td>
<td>141</td>
<td>209</td>
<td>96.5</td>
</tr>
<tr>
<td>Bilirubin (mcmol/l)</td>
<td>25</td>
<td>25</td>
<td>10.93</td>
</tr>
<tr>
<td>Cholesterol total (mmol/l)</td>
<td>1.065</td>
<td>0.40</td>
<td>1.33</td>
</tr>
<tr>
<td>α2 macroglobulin (g/l)</td>
<td>1.98</td>
<td>0.74</td>
<td>2.085</td>
</tr>
<tr>
<td>Acid hyaluronic (mcg/l)</td>
<td>610</td>
<td>767</td>
<td>147</td>
</tr>
<tr>
<td>Apolipoprotein A2 (g/l)</td>
<td>0.87</td>
<td>0.32</td>
<td>1.09</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>205</td>
<td>253</td>
<td>132</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>26.89</td>
<td>5.7</td>
<td>30.86</td>
</tr>
<tr>
<td>TP non AVK</td>
<td>55.84</td>
<td>15.15</td>
<td>73.74</td>
</tr>
<tr>
<td>Haemoglobin (g/l)</td>
<td>10.15</td>
<td>1.88</td>
<td>11.24</td>
</tr>
<tr>
<td>AST (UI/l)</td>
<td>63.61</td>
<td>27.98</td>
<td>57.41</td>
</tr>
<tr>
<td>LOK</td>
<td>0.984</td>
<td>0.07</td>
<td>0.9295</td>
</tr>
<tr>
<td>Hepascore</td>
<td>1</td>
<td>0</td>
<td>0.962</td>
</tr>
<tr>
<td>MELD</td>
<td>12.16</td>
<td>3.24</td>
<td>10.45</td>
</tr>
<tr>
<td>Child Score #</td>
<td>8.11</td>
<td>1.32</td>
<td>7.69</td>
</tr>
<tr>
<td>FORNS #</td>
<td>11.06</td>
<td>1.60</td>
<td>9.77</td>
</tr>
<tr>
<td>Spleen size #</td>
<td>14.32</td>
<td>2.26</td>
<td>12.72</td>
</tr>
<tr>
<td>Fibroscan results (Kpa)</td>
<td>57.88</td>
<td>15.48</td>
<td>48.44</td>
</tr>
</tbody>
</table>

Table 2. AUC of markers with statistical significance for the existence of high-risk esophageal varices
Comparison of the predictive accuracy of clinically significant portal hypertension between the Baveno criteria for hyaluronic acid, TP and spleen size

Screening using the Baveno VI criteria would have been possible in all patients, even when the value of one of the biomarkers is missing. These compound criteria (decompensated cirrhosis, platelets less than 150,000 / mmc or Fibroscan over 20 kPa) had an NPV of 0.75 and a PPV of 0.486 for high-risk esophageal varices.

<table>
<thead>
<tr>
<th>VALUE</th>
<th>SENSITIVITY/ SPECIFICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HYALURONIC ACID</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;169 mcg/l</td>
<td>Maximal sensitivity</td>
</tr>
<tr>
<td>&gt;1400 mcg/l</td>
<td>Maximal specificity</td>
</tr>
<tr>
<td><strong>TP</strong></td>
<td></td>
</tr>
<tr>
<td>&gt; 82%</td>
<td>Maximal sensitivity</td>
</tr>
<tr>
<td>&lt;60%</td>
<td>Maximal specificity</td>
</tr>
<tr>
<td><strong>SPLEEN SIZE</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;8 cm</td>
<td>Maximal sensitivity</td>
</tr>
<tr>
<td>&gt;17.5 cm</td>
<td>Maximal specificity</td>
</tr>
</tbody>
</table>

Table 3. Cutoffs for maximals sensitivity and specificity for the identified biomarkers

Hyaluronic acid, with a cut-off of 361.5 mcg / l (maximum amount of specificity and sensitivity) had PPV at 0.70 and NPV at 0.88. Compared to the Baveno criteria, hyaluronic acid correctly reclassifies 11 (26%) patients. Incorrectly classifies 2 (4.9%) of patients and does not change the classification in 28 patients (68.3%).

The size of the spleen, with a cut-off of 13.5 cm (the maximum amount of specificity and sensitivity) has VPP at 0.40 and VPN at 0.785. Compared to the Baveno discriminant, the size of the spleen correctly reclassifies 1 patient (2.5%). Incorrectly classifies 3 (7.5%) of patients and does not change the classification to 37 (90%).

The TP, with a cut-off at 59.5% (maximum amount of sensitivity and specificity), has VPP at 0.722 and VPN at 0.74. Compared to the Baveno discriminant, TP correctly reclassifies 8 patients (19.5%). Incorrectly classifies 2 (4.9%) of patients and does not change the classification to 31 (75%).

**Multivariate analysis.** A binary regression was performed to evaluate the effect of TP, spleen size and hyaluronic acid on the probability of esophageal varices with a high risk of bleeding. The logistic model was statistically significant
with the value of Chis $q^2 21,831$ with $p < 0.0001$. The size of the spleen and hyaluronic acid had an independent contribution to the prediction value of the model. Odds ratios for an increase in the unit of measurement of spleen size (cm) and hyaluronic acid (mcg / l) were $1.494 (1.002-2.227; p = 0.049)$ and $1.004 (1.001-1.007; p = 0.014)$, respectively. For each $50$ mcg / l increase in hyaluronic acid, the OR is $1.225 (1.042-1.440; p = 0.144)$.

**Maximum sensitivity / specificity model for high-risk esophageal varices**

The continuous model had a predictive value estimated by an AUROC of $0.88$ in the derivation cohort. A categorical model with categories given by absolute specificity - maximum specificity and absolute sensitivity for hyaluronic acid, TP and spleen size had an AUC of $0.926$ with $95\%$ CI of $0.851-1$ and $p < 0.001$

<table>
<thead>
<tr>
<th>CUT OFF</th>
<th>SENSI</th>
<th>SPEC</th>
<th>PPV</th>
<th>NPV</th>
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</thead>
<tbody>
<tr>
<td><strong>FIBROSCAN</strong></td>
<td>20Kpa</td>
<td>1</td>
<td>0.3</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>PLATELETS</strong></td>
<td>150/l</td>
<td>0.83</td>
<td>0.27</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>BAVENO CRITERIA</strong></td>
<td>0.94</td>
<td>0.14</td>
<td>0.5</td>
<td>0.75</td>
</tr>
<tr>
<td><strong>TP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MAX SENSITIVITY / SPECIFICITY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TP MAX SENSITIVITY</strong></td>
<td>59.5%</td>
<td>0.68</td>
<td>0.77</td>
<td>0.72</td>
</tr>
<tr>
<td><strong>HYALURONIC ACID</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MAX SENSITIVITY / SPECIFICITY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HYALURONIC ACID MAX SENSITIVITY</strong></td>
<td>361mcg/dl</td>
<td>0.89</td>
<td>0.68</td>
<td>0.70</td>
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<td><strong>SPLEEN SIZE</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>MAX SENSITIVITY / SPECIFICITY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SPLEEN SIZE MAX SENSITIVITY</strong></td>
<td>13.5cm</td>
<td>0.84</td>
<td>0.5</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>8cm</strong></td>
<td>1</td>
<td>0</td>
<td>0.46</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 4. Maximum sensitivity / specificity model for high-risk esophageal varices

Figure 2 Maximum sensitivity /specificity AUC model for high-risk esophageal varices
3. DISCUSSION

Our paper shows that in non-viral portal cirrhosis, portal hypertension and high-risk esophageal varices are difficult to predict with classical instruments. Easily available biomarkers such as hyaluronic acid, spleen size and prothrombin time may be useful in this role.

The BAVENO VI Consensus on portal hypertension proposed an endoscopic screening for esophageal varices in cirrhotic patients using 3 non-invasive categorical discriminants: decompensated cirrhosis, liver stiffness and platelet count [18]. These 3 biomarkers are extracted from studies with large populations of viral hepatitis. In our study with a population of cirrhotic patients with steatosis origin, both alcoholic and dysmetabolic, the 3 BAVENO discriminants, alone or in combination, remain relatively sensitive but have a very low specificity for high risk esophageal varices.

Liver stiffness measurements were possible in only 22 of the 41 patients. In these patients, hepatic stiffness was predictive for high-risk esophageal varices, with an AUC of .708, with a sensitivity of 100% but the specificity was only 30%. We considered that measurement failure was due to objective factors (ascites, obesity, wall edema) in 9 patients, while in 12 patients compliance was its main driver. The availability of Fibroscan or other liver stiffness measurement techniques is somewhat limited, operator dependent, and time consuming [19].

Thrombocytopenia was a poor predictor of high-risk esophageal varices, as it was present in 31 of the 41 patients. Its etiology in cirrhosis is complex, with an expected prevalence of 64-84%. Alcohol consumption but also vitamin and protein deficiencies in some cirrhotic patients are known to have a negative impact on platelet counts[20-22], so this result was expected in our population. In our study, applying the BAVENO VI criteria for hypertension screening would have generated a screening endoscopy in almost all patients and still failed to identify one patient with high-risk esophageal varices.
Patients with cirrhosis related to steatosis have different hematological, biochemical and hepatic morphological and functional properties when compared to patients with hepatic diseases of other origins. The usefulness of some biomarkers derived from viral cirrhosis in this context seems to be limited. We identified 3 different biomarkers, which are good for excellent predictors of portal hypertension and high-risk esophageal varices in steatosis-related disease: hyaluronic acid, TP, and spleen size.

Liver fibrosis is a dynamic, evolving process and appears to be the main driver of portal hypertension. Hyaluronic acid, a known marker of extracellular matrix abnormalities that occur in cirrhosis, is also a potential marker of portal hypertension. Its role in the prediction of portal hypertension was evaluated in 1991. There was no correlation between hyaluronic acid values and portal hypertension, but the level for portal pressure was set at 5 mmHg, well below the accepted value of 10 mmHg for clinical significance in a zone of uncertainty[23]. It was never tested against hard outcomes like variceal hemorrhage, but it has previously been associated with the risk of developing esophageal varices in viral cirrhosis. We found that hyaluronic acid is an excellent predictor for the existence of high-risk varices.

Prothrombin time, a widely used hemostasis biomarker in cirrhosis, is not predictive of cirrhosis coagulopathy, but appears to be a predictive marker of variceal bleeding. This is probably due to its discriminatory capacity for disease severity and for the identification of esophageal varices with bleeding risk[24]. We found it to be a good predictor in our population.

Spleen size, a readily available morphological marker of cirrhosis, has already been tested and proven to be an interesting biomarker - alone or in combination with platelet counts - for predicting high-risk esophageal varices in cirrhosis of various etiologies. In our study, spleen size, but not platelet count, is correlated with high-risk esophageal varices.

We also tested several multiparametric scores of liver fibrosis or disease severity. Of these, HEPASCORE (versus FORNS, LOK, CHILD and MELD) was
the best predictor of high-risk esophageal varices, but its discriminative power was outweighed by hyaluronic acid.

Partly due to "real life" conditions, our study has some limitations. Liver histology was rarely available, so steatosis was probable (estimated radiologically and on clinical context) and the diagnosis of cirrhosis was mainly based on non-invasive tests. Measurement of liver stiffness was performed in an outpatient setting in our study. The size of our study probably caused us not to identify some predictors that are traditionally associated with cirrhosis and portal hypertension.

5. CONCLUSIONS

• Advanced liver disease suffers from pathological, biological, imaging, and clinical differences depending on its etiology.
• Variceal hemorrhage is a formidable complication of cirrhosis and requires early interventions, within a window of opportunity to limit its occurrence.
• Early interventions are possible only by accurately identifying patients at risk for clinically manifest portal hypertension.
• Most previous studies evaluating advanced liver disease and portal hypertension are performed in populations of patients with viral cirrhosis.
• BAVENO VI portal hypertension screening criteria appear to be oversensitive for patients with an alcoholic or dysmetabolic etiology of cirrhosis and elastometric evaluation is difficult in these conditions.
• Hyaluronic acid, prothrombin time and spleen size seem to allow a non-invasive stratification comparable in performance and at the same time additive to the BAVENO VI criteria in this context.
• The identification of biomarkers specific to the particularities of each patient will allow a personalization of the medical care.
• We will try to identify, in the context of steatohepatitis, other biomarkers that characterize as accurately as possible cirrhosis and portal hypertension.