Bio-markers of hepatic fibrosis: a new diagnostic frontier

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Summary

Although liver biopsy still represents the reference standard for the evaluation of disease progression in chronic liver diseases, a distinct change in clinical practice is currently occurring and the tendency to substitute liver biopsy with surrogate measures (defined in their complex "non-invasive methods") has grown to a level of complexity that needs clarification and guidance. Aim of this article is to provide an overview on the proposed non-invasive diagnostic methodologies, particularly serum biomarkers, and their possible integration with the standard invasive procedures for the evaluation of disease progression, i.e. liver biopsy and the measurement of portal pressure.

Key-words: **Fatty liver, liver fibrosis, non-invasive assessment, serum markers, transient elastography**.

Riassunto

Biomarcatori di fibrosi epatica: nuove frontiera diagnostica

Sebbene la biopsia epatica rappresenti ancora lo standard di riferimento per la valutazione della progressione delle malattie croniche del fegato, si sta facendo strada una diversa pratica clinica, e la tendenza a sostituire la biopsia epatica con misure surrogate (definite nel loro complesso "metodi non invasivi") è cresciuta a tali livelli di complessità che chiarimenti e linee guida sono ormai necessari. Scopo di questo articolo è fornire una panoramica sulle metodologie diagnostiche attualmente disponibili, con particolare riferimento ai biomarcatori sierici, e alla loro possibile integrazione con le classiche procedure invasive per la valutazione della progressione della malattia, come la biopsia epatica e la misura della pressione portale.

Progressive fibrosis and cirrhotic transformation of liver tissue are common pathological outcomes of most Chronic Liver Diseases (CLD). Although the histopathological analysis of liver tissue still represents the reference standard for the evaluation of disease progression in CLD, a distinct change in clinical practice is currently occurring and the tendency to substitute liver biopsy with "non-invasive methods" has grown to a level of complexity that needs clarification and guidance. Overall, the main clinical need is not to substitute liver biopsy but rather to have the possibility of selecting by non-invasive means patients with significant or advanced fibrosis to be subjected to liver biopsy for an adequate staging of the disease.

The introduction and the evaluation of different non-invasive measures for assessing disease progression in CLD is based on the key limitation of using liver biopsy as a reference standard. Indeed, several limitations of liver biopsy, and particularly the fact that a single bioptic cylinder is representative of no more than 1/50.000 of the whole liver, make a fair comparison with a serum marker or Liver Stiffness Measurements (LSM) rather difficult. This also considering that standard histopathological analysis may have the same difficulty of non-invasive estimates in discriminating adjacent stages of fibrosis, i.e. F2 vs F1 or F3 vs F2[1,2]. To minimize these limitations, it is absolutely important that histopathological staging is assessed with all the proposed recommendations concerning the size of the biopsy and the number of analyzed portal tracts[1,3] and

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that the non-invasive methodology (blood test, LSM etc.) is tested within a reasonable time from the liver biopsy, ideally within 24 hours and certainly not within 3-6 months as often reported. In addition, there is need for amelioration and further development of the current histopathological scoring systems. For example, efforts should be directed at staging fibrosis beyond stage F4 since the current system makes no distinction between initial cirrhosis (i.e. thin bridging fibrous septa, with limited or no neo-angiogenesis, surrounding large parenchymal nodules) and advanced and end-stage cirrhosis where the tissue angio-architecture is largely altered[4].

Measurement of the Hepatic Vein Pressure Gradient (HVPG), currently employed for the evaluation of portal hypertension, has been suggested as a reliable end-point to assess the therapeutic benefit of antiviral therapy in patients with advanced hepatic fibrosis due to chronic HCV infection[5-9]. In the absence of significant fibrotic evolution, HVPG does not exceed 5 mmHg, whereas a gradient of more than 5 mmHg is always associated with significant fibrosis. Therefore, when considering to treat patients with advanced fibrosis with a HVPG in the range between 5 and 10 mmHg, measurement of HVPG could provide relevant indications about improvement, stabilization or worsening within the stage of compensated cirrhosis. In view of the fact that, within the range 5-10 mm Hg, portal hypertension is a direct consequence of the fibrotic/cirrhotic transformation of liver tissue, measurement of HVPG could represents a reference standard superior to liver biopsy in advanced stages, although this is still controversial and needs to be further substantiated in prospective studies including a larger number of patients. Overall, it appears that the major limitation of HVPG measurement relies on logistics: it is expensive, requires a dedicated setting and very experienced operators and hence is available only in specialized centres.

A large scientific and commercial investment has been made in the past 10 years in order to develop serum markers able to predict the fibrotic stage of CLD. Among the proposed markers, some reflect alterations in hepatic function but do not directly reflect extracellular matrix (ECM) metabolism: "indirect markers". Others are directly linked to the modifications in extracellular matrix turnover occurring during fibrogenesis: "direct markers"[10-27]. In most of the studies so far published, the AUC (area under the curve) for the ROC (receiver operating characteristic) curve is employed as a measure of test performance, with optimal values being as close as 1.0 as possible. Nevertheless, the reported median AUC in differentiating mild/no fibrosis and significant fibrosis in validation populations is around 0.77 which is far away from high diagnostic accuracy. However, all tests show an improved performance when the end-point is to differentiate cirrhosis/non cirrhosis with median AUC in validation sets of approximately 0.87. In a recent modelling analysis including the majority of the proposed serum marker panels, Parkes and co-workers showed that when positive and negative predictive values (PPV and NPV, respectively) threshold of 90% and 95% were considered, liver biopsy could have been correctly avoided only in an average 35% of the study population, with 20% of patients misclassified and the remaining impossible to classify because of intermediate values[28].

Overall, it is very important to note that, although direct, indirect and combined serum marker systems measure rather different biomarkers, they are all characterized by an AUC for the ROC clustering around 0.85. As already pointed out it is likely that the explanation of this diagnostic equivalency lies in the inaccuracy of liver biopsy as reference standard either in absolute terms or relative to the lack of adequate standards in the so far performed validation studies[29]. A relevant interpretation problem concerns the different spectrum of fibrosis stages ("spectrum bias") that accounts for most of the heterogeneity between studies. For example, if a study is over-represented in extreme stages (i.e. F0 and F4) its specificity and sensitivity will be automatically higher than in a study including only adjacent stages. Therefore, sensitivity and/or standardization analysis should be performed according to these differences in stage prevalence defining advanced (i.e. ≥ F3) and non advanced (i.e. ≤ F2) fibrosis.

A better definition of serum tests could derive from their evaluation in prospective studies employing a combination of different tests. Along these lines, Sebastiani and coworkers reported that a stepwise combination of different algorithms (APRI, Fibrotest, Forns' index) in cohorts of patients with chronic C (HCV) or B (HBV) hepatitis may reduce the need for liver biopsy in 50-70% and 50-80% of cases, respectively[30,31]**.** Moreover, Leroy and co-workers prospectively compared six non-invasive scores for the diagnosis of liver fibrosis in chronic HCV hepatitis[32]. They found that the best combination (including MP3, Fibrotest and APRI) could select one-third of patients with either absence of significant fibrosis or presence of advanced fibrosis with more than 90% certainty. Therefore, at the present stage of development, we can conclude that the diagnostic accuracy of systems employing serum biomarkers has been proved useful for the detection (or exclusion) of significant fibrosis or cirrhosis mainly in patients with chronic HCV infection. However, it is rather clear that these tests may reduce but not eliminate the need of liver biopsy and that platelet count per se allows the exclusion of cirrhosis with a fairly similar degree of accuracy[33,34].

In recent years, Non Alcoholic Fatty Liver Disease (NAFLD) has become a major clinical entity and is indeed associated with several interpretative issues, particularly the definition of the disease stage, i.e. the possible evolution towards advanced fibrosis and cirrhosis. NAFLD encompasses a spectrum of diseases ran-

ging from simple steatosis with or without non-specific inflammation, to a more severe entity, Nonalcoholic Steatotic Hepatitis (NASH), which is associated with fibrosis and carries a significant risk to progress to cirrhosis and its complications[35]. The diagnosis of NASH is essentially based on histopathology and it is more dependent on liver biopsy than other CLD. This represents a key clinical problem since patients with NASH and fibrosis require a close follow-up and, given the extremely high prevalence of this condition in the general population, the possibility of a non-invasive assessment would have an important impact on both public health and health economics.

Although sophisticated and extensively validated diagnostic algorithms to be employed in patients with NAFLD are not currently available, a number of cross-sectional studies evaluating patients with NASH and fibrosis has allowed the identification of clinical and biochemical parameters associated with advanced stages of fibrosis in patients with NAFLD. While some indicators are in common with other CLD, it is evident that parameters related to the clinical features of the metabolic syndrome have a major impact also on the fibrotic evolution of NAFLD. Interestingly, age and insulin-resistance, that almost invariably emerged as risk factors in cross-sectional studies[36,37] correlated less strictly with fibrosis progression in longitudinal studies[38,39], reflecting the complexity of understanding fibrosis dynamics in this CLD and the importance of longitudinal studies in general. The role of autoantibodies recognizing adducts with oxidative stress-related products recalls data previously described in alcoholic liver disease[40], while an increase in ferritin levels has been interpreted as a proxy of inflammatory activity rather than a marker of iron overload.

A small number of studies have provided performance data for tests that identify fibrosis in patients with NAFLD. Importantly, only in a few studies[37,41] the results of the training set were confirmed in an independent validation set. Moreover, interpretation of the available data is not always easy, particularly because these series often report on small numbers of patients and the assessment of fibrosis stage varies across different reports. It is also important to note that performance of the tests varies based on the prevalence of the severity of fibrosis in the population tested and this further hampers the possibility to extrapolate the results. This is particularly important when considering that patients with NAFLD may be seen in settings (e.g. diabetes or obesity clinics) where the prevalence of advanced fibrosis is largely lower than in a hepatology referral centres. Of note, the recently reported NAFLD Fibrosis Score (NFS)[42] includes the presence or absence of diabetes among other parameters. Overall, it is quite clear that also the non-invasive serum markers proposed for NAFLD will allow to identify or exclude patients with severe fibrosis, although a large proportion of the population is likely to fall in an undetermined area. Regardless, the key diagnostic tool in NAFLD would be a non-invasive test able to differentiate the presence of NASH from bland steatosis. Along these lines several tests have been proposed and await adequate validation. Some tests are derived from those already proposed for predicting the stage of fibrosis with the inclusion of clinical parameters typical of NAFLD, i.e. the NashTest[43,44]. An alternative and original approach is represented by the evaluation in 44 patients of plasma caspase-3-generated cytokeratin-18 fragments, a biomarker of hepatocyte apoptosis[45]. Levels of cytokeratin-18 fragments were able to identify patients with NASH as compared to those with bland steatosis with remarkably high specificity and acceptable sensitivity. Conversely, the algorithm proposed by Bedogni et al. relies on typical NAFLD parameters like BMI, waist circumference, and triglycerides[46], but was designed simply to detect steatosis in the general population and not to identify NASH.

In conclusion, the present status of development of different non-invasive tools testifies the large effort for a better clinical definition of the fibrogenic progression of chronic hepatitis C as well as other liver fibrogenic disorders. Some major considerations arise from the experience so far accumulated. First, all non-invasive methodologies are characterized by a sufficient to excellent diagnostic accuracy for the detection (or exclusion) of advanced fibrosis and cirrhosis and none is able to allow a step-wise follow-up of the fibrogenic evolution of CLD according to the existing histopathological staging systems. In other words, due to the absence of a true gold standard, the definition of a 90% diagnostic accuracy remains a goal for the future. In addition, none of the currently available tests has a well defined prognostic value such as the prediction of decompensation or death. Second, due to the "spectrum bias" and the possible causes of discordance with the histopathological assessment, the applicability of the different proposed cut-off values in clinical practice is presently hazardous. Regardless, it is more and more evident that a rational and prudent use of the proposed methodologies will reduce the need of liver biopsy in a significant percentage of patients and represents a diagnostic advantage.

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