Serum biomarkers for the non-invasive diagnosis of liver fibrosis: the importance of being validated

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Chronic liver diseases (CLDs), including chronic hepatitis C and B, alcoholic and non-alcoholic fatty liver diseases, represent a major cause of morbidity and mortality worldwide. The progressive accumulation of fibrosis in the liver characterizes the natural history of CLDs and represents the hallmark of the evolution towards hepatic cirrhosis and its end-stage complications (1). Staging of liver fibrosis is essential to define prognosis and management of CLDs. Classically, two stages of liver fibrosis are considered relevant by guidelines and clinicians since they significantly modify the management of patients: significant fibrosis, as defined as $\geq F2$ by METAVIR classification, and cirrhosis, as defined as F4 by METAVIR. The former is considered a definitive indication for antiviral treatment in chronic viral hepatitis B and C, the latter requires a specific follow-up for the risk of hepatocellular carcinoma and esophageal varices (2, 3). Liver biopsy has long been regarded as the gold standard of reference for the staging of fibrosis. However, it has major drawbacks including cost, side effects and risk of underestimation of liver fibrosis stage if sampling is inadequate (4). Accordingly, in the last 10 years many efforts have been dedicated by the researchers towards the identification of surrogate markers able to provide a non-invasive assessment of fibrosis in the liver. A number of serum biomarkers have been proposed for the non-invasive diagnosis of liver fibrosis (1). These can be broadly classified in three main groups: 1) direct markers; 2) indirect markers; and 3) patented tests. Direct markers are fragments of the liver matrix components, such as hyaluronic acid (HA) and products of collagen metabolism, produced by hepatic stellate cells during the fibrotic process. This group of biomarkers reflects the metabolism of hepatic extracellular matrix and has a pathophysiologic rationale. However, a limitation to the clinical use of direct markers of liver fibrosis is that they are not routinely available in all hospital settings. In contrast, indirect markers are biochemical parameters measurable in the peripheral blood that are routinely performed in patients with CLDs. They are indirect expression of liver damage and are not directly involved in the fibrotic process. These include molecules synthesized, regulated or excreted by the liver, such as clotting factors, bilirubin, transaminases and albumin. More recently, direct and indirect biomarkers have been combined in patented tests with the aim of achieving a higher diagnostic accuracy than the single parameters. Among this last group of serum biomarkers, five patented tests have been proposed and investigated in CLDs, including Fibrotest, Hepascore, Fibrometer, Enhanced Liver Fibrosis (ELF) test and Fibrospect (5–9). Interestingly, some studies have suggested that patented tests may perform better than simple, indirect serum biomarkers (10). However, indirect biomarkers are routinely performed in patients with CLDs, do not require any additional cost and they are easily accessible in clinical practice.

In this issue of *Clinical Chemistry and Laboratory Medicine*, Guechot and colleagues proposed an independent validation of ELF score in a large cohort of patients with chronic hepatitis C. The authors report an area under the curve of 0.78 for significant fibrosis and of 0.85 for cirrhosis that is somehow in line with previous studies (11). Moreover, they showed that the ELF score performed better than HA and slightly worse as compared to Fibrotest and Hepascore.

The validation of patented serum biomarkers is a critical issue for their widespread use in clinical practice. Indeed, the validation of a patented test, independently from the group who commercialized it, increases the credibility in the test itself. The concept of validation should encompass several aspects that become even more critical when dealing with patented serum biomarkers (Table 1).

First, a patented biomarker should be compared with simple, economic and extensively validated biomarkers, such as AST-to-Platelet Ratio Index (APRI), and it should demonstrate a clear advantage in terms of diagnostic accuracy (16).

Second, since most of the serum biomarkers have been developed and investigated in chronic hepatitis C, dedicated validation studies in other etiologies of CLDs should be carried out (1). Indeed, each etiology of CLD presents with specific pathogenesis, natural history and associated comorbidities. For example, when considering chronic hepatitis C and chronic hepatitis B, the former has specific associated comorbidities, such as steatosis and diabetes; the latter is characterized by a more vigorous necroinflammation (12).

Third, a careful evaluation of the risk factors for error of a patented biomarker and their frequency should be carried out. Both the clinician and the laboratory professional should be aware of the conditions that may affect the result of the serum biomarker and that may render the provided information unreliable. Among the patented serum biomarkers, Fibrotest has been extensively investigated as regards to conditions that alter its result, including Gilbert syndrome, systemic inflammation, hemolysis, extraepatic cholestasis (1).

Fourth, serum biomarkers should be specifically validated in special populations that are particularly frequent and in
which the clinical management is significantly influenced by the stage of liver fibrosis, including hepatitis C virus (HCV) patients with normal transaminases and HCV patients co-infected with human immunodeficiency virus (HIV). Indeed, it has been suggested that serum biomarkers may have a significantly lower performance in HCV patients with persistently normal transaminases (17).

Fifth, application of serum biomarkers for routine use in clinical practice could be limited by the lack of adequate diagnostic accuracy. Very often, serum biomarkers, including the patented ones, did not overcome 80% diagnostic accuracy for the diagnosis of significant fibrosis when used alone. Recently, some studies have reported that the accuracy of serum biomarkers may significantly improve when they are combined in diagnostic algorithms (13, 15, 18).

With such a large study population, Guechot et al. could have addressed many of the issues related to an exhaustive validation of ELF score, including the direct comparison with simple biomarkers, such as APRI, the dissection of risk factors for error, the investigation of the diagnostic accuracy in HCV patients with normal transaminases, and the development of algorithms combining ELF score and other biomarkers for application in clinical practice. Importantly, the concept of combining unrelated serum biomarkers or a serum biomarker and a widespread ultrasound elastometry system, named Fibroscan, has recently received large consensus by guidelines and opinion leaders (2, 13, 14, 19).

Furthermore, when dealing with patented biomarkers, analytic conditions, such as standardization of reagents and analyzers according to manufacturer’s recommendations, should be taken into account.

Finally, even with its limitations, liver biopsy remains the gold standard of reference for the staging of liver fibrosis in CLDs (4). In this view, a rational approach could be to implement the use of the most validated serum biomarkers as screening tests and to limit the use of liver biopsy in case of an unreliable result or according to the clinician’s judgment.

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**References**


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