Battle in Search for Simple Reliable Non-invasive Marker of Liver Fibrosis

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The development of hepatic fibrosis is an important landmark in the natural history of liver disease as its subsequent evolution to cirrhosis is associated with ominous and often irreversible changes in structure, function, and hemodynamics of the liver along with propensity to develop malignancy. Hence, there is a definite need for non-invasive markers that can accurately assess the extent of fibrosis and cirrhosis in an effort to prognosticate the liver disease. Till now, liver biopsy has been the 'gold standard' for assessing the severity of necroinflammatory activity and liver fibrosis, but it is invasive and sometimes associated with rare but serious complications, including bleeding, pneumothorax, and procedure-related death.^{1,2} Its inability to give targeted tissue (being a blind procedure) in addition to not infrequent sampling error along with well-known intraobserver and interobserver variability in histological interpretation has made liver biopsy *a less than a perfect* 'gold standard' for the diagnosis of liver fibrosis^{2,3} primarily due to the lack of better alternatives. These limitations have propelled the need for search of new non-invasive approaches for the diagnosis of liver fibrosis. Ideally, a non-invasive marker of liver fibrosis should be liver-specific, easy to perform, reliable, reproducible, and inexpensive. It should, in addition, be accurate not only for the staging of fibrosis, but also for monitoring disease progression. For all of these very reasons, there is an urgent need to develop and validate simple, reproducible, non-invasive tests that accurately distinguish cirrhosis from noncirrhotic patients and can determine the stage and grade of the disease based on the extent of fibrosis. Biomarkers of fibrosis are commonly divided into direct and indirect markers. Direct markers are fragments of the liver matrix components produced by hepatic stellate cells during the

process of extracellular matrix remodeling. Indirect markers include molecules released into the blood due to liver inflammation, molecules synthesized/regulated or excreted by the liver, and markers of processes commonly disrupted due to hepatic dysfunction. Current non-invasive methods for the assessment of liver fibrosis range from simple serum biomarker assays to advanced imaging techniques such as transient elastography and magnetic resonance imaging (MRI). Though far from ideal, the non-invasive methods that have the strongest clinical and histopathological correlates are FibroScan elastometry and two serum-based tests, aspartate aminotransferase/platelet ratio index (APRI) and FibroTest.⁴

In this issue of the *Journal of Clinical and Experimental Hepatology*, Sethi et al⁵ have made an elegant effort to further strengthen the role of non-invasive marker in the evaluation of patients with cirrhosis vs no cirrhosis. They evaluated circulatory endothelial cells (CEC), a marker of vascular injury, as a new tool for differentiating patients with significant fibrosis and cirrhosis compared with those without it. For this study, they enrolled 53 patients with cirrhosis, 20 matched healthy controls and 9 patients with noncirrhotic liver disease. They found that median CEC counts were significantly higher in patients with cirrhosis (62 cells/4 mL, interquartile range [IQR]: 43.5–121) as compared with controls (31 cells/4 mL, IQR: 22.2–40). The CEC/PC was also significantly elevated in cirrhotics (0.69, IQR: 0.39–1.48) compared with controls (0.12, IQR: 0.09–0.20) and noncirrhotics (0.21, IQR: 0.08–0.43). Receiver operator characteristic (ROC) analysis revealed that CEC cutoff value of ≥ 37 cells/4 mL showed sensitivity of 81% and specificity of 75% for differentiating cirrhosis from controls (AUC: 0.80; 95% confidence interval [CI] 0.67–0.91). Circulatory endothelial cells/PC ratio cutoff value of ≥ 0.23 showed sensitivity of 91% and specificity of 82% (area under the curve [AUC]: 0.92; 95% CI 0.83–0.99). The APRI cutoff value of ≥ 0.4 showed sensitivity of 94% and specificity of 85% for differentiating cirrhosis from control patients (AUC: 0.96; 95% CI 0.90–1.0). They concluded that a product of CEC and APRI, termed CAPRI (CEC–APRI), effectively distinguished patients with cirrhosis from controls; with cutoff value of ≥ 12.7 , showing higher sensitivity (98%) and specificity (85%) (AUC: 0.98; 95% CI 0.96–1.0) than the time tested APRI alone.

Cirrhosis and portal hypertension are characterized by marked anatomical and functional abnormalities in the hepatic and systemic circulation including vascular endothelial injury and characterized by prominent vascular changes involving the endothelium, including angiogenesis,

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Abbreviations: APRI: AST/platelet ratio index; AUC: area under the curve; CAPRI: CEC with APRI; CEC: circulating endothelial cell; EGD: esophagogastroduodenoscopy; IQR: interquartile range; PC: platelet count; ROC: receiver operator characteristic

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sinusoidal vascular distortion, hyperdynamic systemic circulation, and intrahepatic endothelial dysfunction.⁶ The precise origin of CEC in patients with cirrhosis and portal hypertension remains to be determined and whether CEC is a marker of portal hypertension or cirrhosis/fibrosis or combination of two is also not clear. We wish the author would have included a group of patients with noncirrhosis with involvement of vascular system like idiopathic non-cirrhotic portal hypertension (INCPH),^{7,8} which is characterized by periportal fibrosis and sclerosis and obliteration of small and medium branches of portal vein involvement of small and medium branches of the portal vein (obliterative portal venopathy) and extrahepatic portal vein obstruction that would have clarified the utility of CEC to already existing indices of fibrosis like APRI. Theoretically, CEC may derive from mature endothelial cells sloughed from the vessel wall in response to vascular injury, or from endothelial progenitor cells, which are bone-marrow-derived and postulated to contribute to vascular repair. Hence, CEC in general may not be a specific marker of cirrhosis.⁹⁻¹¹ Recent studies indicate that CEC are increased in the peripheral blood of patients with various forms of vascular injury and endothelial damage.¹²⁻¹⁴ In fact, CEC have been identified as a specific, non-invasive surrogate biomarker of vascular damage in specific cardiovascular diseases. In a pilot study, Abdelmoneim et al¹⁵ evaluated CEC in 15 cirrhotic patients and 15 controls and ROC analysis revealed that CEC cutoff of 42 cells/4 mL showed sensitivity of 87% and specificity of 74% for differentiating cirrhosis from controls (AUC: 0.74), while CEC/PC ratio at 0.21 showed sensitivity of 100% and specificity of 73% (AUC: 0.89). So different cutoff for CEC and CEC/PC revealed different sensitivity and specificity, which is true for other non-invasive markers in different studies, and does not fit under the criteria of an ideal non-invasive marker.

In this article, the authors have compared CEC with APRI and found it to be as sensitive as APRI. They found high sensitivity and specificity of APRI in differentiating cirrhosis with no cirrhosis. However, APRI itself has not shown to be a useful non-invasive marker of fibrosis. In a meta-analysis by Jin et al¹⁶ who systematically reviewed the performance of the APRI in predicting significant fibrosis and cirrhosis in hepatitis B-related fibrosis, for significant fibrosis an APRI threshold of 0.5 was 84% sensitive and 41% specific and at the cutoff of 1.5, the sensitivity and specificity were 49% and 84%, respectively. For cirrhosis, an APRI threshold of 1.0–1.5 was 54% sensitive and 78% specific and at the cutoff of 2.0, the sensitivity and specificity were 28% and 87%, respectively. Lin et al¹⁷ in HCV-related liver disease found that AUROC of the APRI for the diagnosis of significant fibrosis, severe fibrosis, and cirrhosis was 0.77, 0.80, and 0.83, respectively. So it would have been better if the authors had compared the CEC test with FibroTest and carried out the evaluation of CAPRI in different stages of fibrosis in this phase of study after their

previously published similar study.¹⁵ We complement the authors of this study for their innovative idea of a newer index called CAPRI to non-invasively predict the presence of cirrhosis. However, its widespread use in clinical practice needs more data and validation in large cohort of patients.

Lastly, the cost issue should be kept in mind in day-to-day practice while searching for better non-invasive marker compared with already existing fibrosis prediction models. The CEC has excellent prediction of cirrhosis and its cost is currently higher than APRI; however, it could be anticipated that the cost of CEC would decrease with wider application owing to increased vendor availability and marketplace competition. Its validation in larger cohort of patients and in subgroup of patients with vascular injury in the form of INCPH, portal vein thrombosis, or Budd–Chiari syndrome needs to be evaluated. Non-invasive investigations, such as various biomarkers, fibrosis scoring panels, and imaging techniques, offer considerable promise in their ability to differentiate no fibrosis vs liver cirrhosis in 80–90% of cases. In fact, most of these tests can primarily distinguish cirrhosis from no or minimal fibrosis. For the diagnosis of significant liver fibrosis ($F \geq 2$), non-invasive tests cannot as yet replace liver biopsy. Therefore, the current utility of non-invasive diagnostics remains limited to pre-screening allowing physician to narrow the population of the patient before definitive diagnosis of liver fibrosis by liver biopsy. With the advent of proteomics and better biomarkers studies coupled with promising functional magnetic resonance spectroscopy studies, a day may not be far away when liver biopsy may be obsolete even to distinguish various stages of fibrosis within the liver. So an unending search for an ideal non-invasive marker is still on and we sincerely hope that such a test will sooner than later see the light of the day.

CONFLICTS OF INTEREST

All authors have none to declare.

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