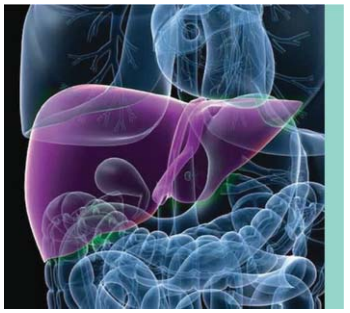

Biomarkers of Liver Fibrosis

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Currently, the only accepted method (gold standard) for the diagnosis of the fibrotic stages of chronic liver disease (CLD) is liver biopsy, to allow histological assessment. Liver biopsy is an invasive investigation associated with a range adverse events (e.g., pain and hemorrhage),[1,2] limiting its serial usage in clinical practice. In addition, its use is further reduced by sampling error[3] and because histology is in effect a surrogate for clinical outcomes.

Over recent years, alternative noninvasive biomarkers for the diagnosis of liver fibrosis have been developed. Initially developed in chronic viral hepatitis, these have since seen their use expanded to include all causes of CLD. Such markers can be divided into indirect ''simple'' markers (e.g., transaminases, gamma-glutamyl transferase, and platelet count), direct ''complex'' markers (e.g., procollagen peptides I/III and type IV collagen), cytokines (e.g., interleukin-10 and transforming growth factor α), and imaging. In this review, we discuss the clinical utility, limitations, and development of noninvasive biomarkers in their use as diagnostic and prognostic tests.

CLINICAL UTILITY OF CURRENT BIOMARKERS IN ASSESSING LIVER FIBROSIS

Indirect ''Simple'' Markers

Indirect markers measure components not directly involved in the fibrosis process. Although having the advantage of being relatively inexpensive and easy to perform, they lack diagnostic accuracy for the detection of hepatic fibrosis. For example, Kayadibi et al.[4] found for the diagnosis of any fibrosis, the sensitivity and specificity of alanine aminotransferase to be 68% and 81%, and for aspartate aminotransferase to be 48% and 83%, respectively. These correspond to a positive predictive value (PPV) in a low-prevalence population (5%) of only 10% and 9%.

Direct ''Complex'' Markers and Cytokines

Direct ''complex'' markers measure components of the fibrosis pathway and are frequently combined as panel markers with perceived improved diagnostic accuracy over individual markers. Currently, cross-sectional data suggest that such biomarkers could be used as an

Abbreviations: APRI, aspartate-to-platelet ratio index; AUROC, area under the receiver operating curves; CLD, chronic liver disease; FIB4, Fibrosis-4 index; MR, magnetic resonance; NAFLD, nonalcoholic fatty liver disease; NFS, nonalcoholic fatty liver disease fibrosis score; NPV, negative predictive value; PPV, positive predictive value; TE, transient ultrasound elastography.

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Potential conflict of interest: Nothing to report.

Received 15 January 2016; accepted 28 March 2016

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alternative to liver biopsy in some patients. For example, Guha et al.[5] present a clinical utility model showing that the enhanced liver fibrosis panel can be used to avoid liver biopsy in the diagnosis of advanced liver fibrosis in 88% of cases, with only 14% of these cases incorrectly avoiding biopsy. However, these figures decline to 48% and 21%, respectively, for the diagnosis of any fibrosis.[5] Comparable accuracy is seen when complex markers are tested in viral hepatitis.[6] A second use of cross-sectional data is for the prediction of liver disease development and prognosis. Kim et al.[7] found patients with nonalcoholic fatty liver disease (NAFLD) fibrosis (determined by the NAFLD fibrosis score [NFS]) had a higher probability of all-cause and cardiovascular death (adjusted hazard ratio 1.69 and 3.46, respectively) compared with those with a low NFS. These results were partially replicated for the simpler biomarkers, aspartate-to-platelet ratio index (APRI) and the Fibrosis-4 index (FIB4), with both associated with increased cardiovascular death and APRI additionally associated with all-cause and diabetes-related death. Angulo et al.[8] had similar findings with NFS, APRI, and FIB4 (but not BARD) associated with all-cause death and all four markers associated with future clinical liver events.

Similarly to direct markers, cytokines have been identified as potential markers of fibrosis because they are involved in the regulation of the inflammatory response to liver cell injury and fibrogenesis. A number of studies have noted raised levels of cytokines in patients with hepatic fibrosis, but few have evaluated their diagnostic accuracy.

Imaging

The future of noninvasive biomarkers is likely to lie in imaging, allowing the assessment of the whole liver, avoiding sampling error and the need for surrogate markers. Although transient ultrasound elastography (TE) is an easily accessible technology, it is subject to operator[9] and subject limitations.[10] For example, in NAFLD, accuracy in high-prevalence (30%) populations is good (PPV 67%, negative predictive value [NPV] 93%), but again there is a notable decline in PPV in low-prevalence (5%) populations (PPV 18%, NPV 99%).[11] It has also been noted that although accuracy is maintained, the optimal cutoff values of TE vary by underlying causative factor.[12] However, magnetic resonance (MR) elastography has excellent accuracy for advanced liver fibrosis[13,14] with the main limitation of requiring additional hardware. Furthermore, novel MR imaging protocols not requiring contrast or additional hardware are now beginning to emerge.[15,16]

DIAGNOSTIC LIMITATIONS OF CURRENT BIOMARKERS OF FIBROSIS

As noted earlier, large numbers of cross studies have been undertaken attempting to validate the use of noninvasive biomarkers in the diagnosis of liver fibrosis resulting in acceptable diagnostic accuracy for advanced fibrosis and cirrhosis (METAVIR F3/4). However, their findings have found very limited use in early and intermediate CLD.

Further methodological concerns with these studies exist: few used a development and a validation cohort with the majority not replicated, they were often small ($n<100$), and spectrum bias limits applicability with the choice of study population typically tertiary care focused. A heavy reliance on area under the receiver operating curves (AUROC) misses the clinical context, with the definition of a good AUROC being relative and not absolute. The optimal diagnostic test accuracy metric is determined by the clinical question.

There have been few longitudinal investigations of serial markers, and studies focused on clinical outcomes (as opposed to histology) are challenging but are now starting to emerge.

DEVELOPMENT OF BIOMARKERS OF NONALCOHOLIC FATTY LIVER DISEASE FIBROSIS

Of significant interest now is the ability to detect CLD in a practical manner in the community. For this reason we need to be clear on the question we want to answer, for example, do we want to detect people with fibrosis or those at risk for fibrosis? Pragmatic population-based screening strategies need to be used, focused on risk factors rather than liver enzymes,[17] and using methods that are easily administered in community settings such as TE.[18]

In the future, researchers need to consider how changes in biomarkers over time are related to CLD and clinical outcomes. These have the potential to be powerful tools, transferable to many different populations. To date, no NAFLD studies have considered delta change; however, techniques are being investigated in hepatitis C virus using both serial serum markers[6] and serial TE.[19]

TABLE 1. COMPARISON OF EXISTING AND EMERGING NONINVASIVE MARKERS OF HEPATIC FIBROSIS

	Liver Biopsy	Indirect "Simple" Markers	Direct "Complex" Markers and Cytokines	TE	MR Elastography
Utility in defining stage of fibrosis	Useful for full spectrum	Most useful for advanced fibrosis	Most useful for advanced fibrosis	Most useful for advanced fibrosis	Most useful for advanced fibrosis
Prediction of clinical outcomes	Hepatocellular carcinoma, varices	Hepatocellular carcinoma	Hepatocellular carcinoma, varices	Hepatocellular carcinoma, varices	No data presently
Access to and utility of serial assessment	Not practical because of invasive nature	Easily accessible Emerging data for utility	Easily accessible Emerging data for utility	Relatively easy access (equipment and experienced operator required) Emerging data for utility	Limited access No data presently for utility
Financial costs*	$1,500 per procedure	Various, $1-$10 per measure	Various, $70-$200 per measure/panel	Capital costs for machine: $60,000 Operational cost: $70 per procedure	Capital costs: >$250,000 Operational cost: $300 per procedure
Reliability	Sampling error (1/50,000th of liver sampled)	Laboratory variability	Typically measured at a central laboratory	Operator variability Reliability reduced in obesity, ascites, liver masses, cholestasis	Limited data available
Performance location	Hospital	Community or hospital	Community or hospital	Community or hospital	Hospital

*Costs obtained from appendix 9 in Crossan et al.[20]

SUMMARY

The optimal use of noninvasive fibrosis biomarkers in NAFLD depends on the setting and question under consideration (Table 1). At present, in secondary care settings there is evidence that some noninvasive biomarkers can be used in the diagnosis of advanced liver fibrosis, avoiding the need for invasive liver biopsy. However, these same markers and cutoffs may not be similarly suited to the identification of CLD and prediction of clinical outcomes in community populations. Furthermore, further study of imaging techniques and serial measures is needed to fully understand the relationship between noninvasive biomarkers and the progression/regression of liver fibrosis in the context of hard clinical outcomes.

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