pISSN 2287-2728
eISSN 2287-285X

CMH

Editorial

http://dx.doi.org/10.3350/cmh.2013.19.2.116
Clinical and Molecular Hepatology 2013;19:116-119

Noninvasive markers: a double-edged sword that stratifies nonalcoholic steatohepatitis

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Keywords: Fatty liver; Cytokeratin 18; Apoptosis; Steatohepatitis; Fibrosis

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Nonalcoholic fatty liver disease (NAFLD) has been recognized as the most common liver disease with an estimated prevalence of 20-30% in the Western world and 16-33% in Korea.[1-3] NAFLD is considered to be the hepatic manifestation of metabolic syndrome, because the mechanism underlying the development of NAFLD has been linked to insulin resistance and metabolic syndrome.[4-6] Therefore, NAFLD is closely related to obesity, dyslipidemia, type II diabetes and coronary artery disease.[7-9]

NAFLD encompasses a broad spectrum of hepatic dysfunction ranging from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma.[10,11] Although NAFL typically follows a benign clinical condition, NASH is a potentially progressive disease given that 25% of patients may develop cirrhosis, complications of portal hypertension, and hepatocellular carcinoma.[12,13] Furthermore, patients with NASH have increased mortality compared to the general population.[14,15] A recent large population study shows that NAFL was not associated with higher mortality risk after a median follow-up of 15 years.[16] Further analysis shows that NAFLD patients without advanced fibrosis do not have a higher mortality risk and mortality increases as fibrosis advances.[16] Therefore, distinguishing between NAFL and NASH with or without fibrosis is clinically important for prognosis and therapeutic interventions.

Although there is past and ongoing research for various noninvasive tests to distinguish NASH from NAFL, liver biopsy remains the reference standard method to diagnose the presence of NASH and assess the severity of liver injury. However, liver biopsies are not easily performed because of several limitations. First, it is an invasive procedure with potentially significant complications occurring with a morbidity and mortality rate of 3% and 0.03%, respectively.[17,18] Second, a percutaneous liver biopsy captures an extremely tiny portion of the liver (~1/50,000 of total mass of the liver), leading to significant sampling variability (25%-40%).[19,20] In addition, the extent of variation from observer interpretation by expert pathologists may be as high as 20%.[21]

Therefore, there is an urgent need to investigate and validate a reproducible noninvasive test that distinguishes NASH from NAFL. Several studies have tried to identify potential noninvasive biomarkers, which are markers of the key pathways believed to be associated with NASH pathogenesis. These pathways include oxidative stress, inflammation, insulin resistance, and apoptosis.[22,23]

Abbreviations:

AUROC, area under receiver operating characteristic curve; CK-18, Cytokeratin 18; CI, confidence interval; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis

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Received : May 10, 2013 / **Revised :** May 15, 2013

High-sensitivity C-reactive protein, plasma pentraxin 3, tumor necrosis factor-alpha, and interleukin-6 were studied, but current results were not sufficiently accurate for clinical use.[24-28] NashTest (Biopredictive Paris, France), which uses a proprietary algorithm, has the area under the receiver operating characteristic curve (AUROC) of 0.79.[29] However, this index has only been validated in the original studies and further validation is needed for clinical implication.

Hepatic apoptosis is suggested to play a critical role in liver injury and progression of NAFLD.[30] Cytokeratin 18 (CK-18) is the major intermediate filament protein in the liver and linked to the morphological changes of apoptosis. In the original study, CK-18 fragments were tested in the livers and plasma for biopsy-proven NAFLD and controls, and caspase-3 generated CK-18 activation in the blood that was an independent predictor of NASH.[31] Subsequently, a multicenter validation study was performed and included 139 patients with biopsy-proven NAFLD from the NASH Clinical Research Network and 150 age-matched healthy controls.[32] The AUROC for NASH was estimated to be 0.83 (95% Confidence Interval (CI) 0.75-0.91).[32] CK-18 levels were an independent predictor of both NASH and severity of NAFLD in this study.[32] CK-18 is the only marker for NASH that has been externally validated in nine studies, enrolling a total of 856 NAFLD patients of various ethnicities, obesity, and diabetes status in a recent meta-analysis.[33] AUROC, sensitivity, specificity are 0.82 (95% CI 0.76-0.88), 0.78 (0.65-0.91), and 0.86 (0.75-0.97), respectively.[33] In addition, CK-18 was found to be the most accurate biomarker for the diagnosis of NAFLD and NASH in combination with fibroblast growth factor 21.[34]

In this study by Kim et al, they conducted a prospective cohort study with 108 patients with biopsy-proven NAFLD from 10 participant centers across Korea to i) identify the useful clinical parameters in a noninvasive approach to distinguish NASH from NAFL and ii) to determine whether these levels would be related to the severity of liver injury in patients with NASH.[35] CK-18 levels had a positive correlation with NAFLD activity score and subtype. The serum CK-18 level was significantly higher in the NAFLD subtype 3 or 4 group (NASH group) than the NAFLD subtype 1 or 2 (NAFL group). A CK-18 cutoff value of 235.5 U/L showed a sensitivity of 69.9%, a specificity of 64.9%, and positive and negative predictive value of 75.5% and 57.1%, respectively, for the diagnosis of NASH. The strength of this multicenter prospective study is that serum CK-18 has potential as a non-invasive biochemical marker to distinguish NASH from NAFL in Korean patients for the first time. In addition, this study provided valuable information that the prevalence of significant fibrosis was 35.2% (38/108) in adults with a mean age of 39. We definitely considered that more patients with more severe disease were included in the study than NAFLD patients from the general population due to the enrollment of liver biopsy-proven NAFLD patients in the tertiary hospital. Currently, to distinguish between NAFL and NASH with or without fibrosis, we needed to use noninvasive markers of fibrosis, which are useful in monitoring, counseling, and in some cases, making treatment decisions in patients with NAFLD in Korea and in the Western world.

In this study, the AUROC of CK-18 was relatively low (0.605) compared to previous studies with a relatively high AUROC up to 0.83 for the diagnosis of NASH.[32,33] An NAFLD activity score consists of the weighted sum of each of the following: steatosis, lobular inflammation, and the presence of hepatocyte ballooning. The major difference between the NAFLD activity score, used as definition in previous studies, and the NAFLD subtype category, used as definition of NASH in this study, is that the NAFLD subtype incorporated fibrosis into its definition.[36] Generally, the original criteria for the NAFLD subtype used in this study does not use commonly compared criteria according to the consensus of the NASH Clinical Research Network Pathology Committee used in other studies.[30,32] Therefore, it is important to carefully consider the differences and similarities among several histologic scoring systems when assessing disease severity, the accuracy of noninvasive markers, and the prognosis of NAFLD.[36,37]

There were noteworthy limitations in this study. To confirm whether CK-18 levels are an independent biomarker of NASH in this study, the statistical significance of CK-18 using multivariate logistic regression analysis might be helpful in drawing a more evident conclusion. In addition, as the author stated, the patients were recruited in a tertiary academic hospital where all the patients had a liver biopsy. Hence, patients with more severe disease activity than NAFLD patients from the general population were included in this cohort, constituting a selection bias.

In conclusion, this study validated serum CK-18 as a biochemical marker to distinguish NASH from NAFL in Korean patients. Because this article has multi-institutional data, it has clinical value for Korean NAFLD patients. To confirm these results, larger validation studies and longitudinal prospective analyses are required in the future.

Conflicts of Interest

The authors have no conflicts to disclose.

 http://dx.doi.org/10.3350/cmh.2013.19.2.116

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