Surrogate markers in non-alcoholic steatohepatitis

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Abstract

Background. Hepatic steatosis with inflammation, inflated hepatocytes, and potential fibrosis defines non-alcoholic steatohepatitis (NASH), which can possibly lead to liver cirrhosis. Although liver biopsy is still the gold standard for diagnosing NASH, numerous non-invasive surrogate markers have been investigated to reduce the need for this invasive technique. In this review we present several currently assessed biomarkers, scores, and indexes in assessing NASH.

Methods. A search in the main medical literature databases was conducted. We searched for observational studies evaluating non-invasive markers, scores, and panels in predicting NASH.

Results. Several proinflammatory markers, inflammation and apoptosis biomarkers, as well as complex models have been studied in predicting NASH. Proinflammatory markers include C-reactive protein, ferritin, tumor necrosis factor-α, interleukin-6, pentraxin-3, and neutrophil extracellular traps. Inflammation and apoptosis biomarkers include cytokineratin-18, adipocytokines, lipid oxidation panels, plasminogen activator inhibitor-1, and products of free radical-mediated oxidation of linoleic acid. Moreover, several studied complex models such as NashTest, NashTest-2, pairing CK18 fragments with other biomarkers such as ALT and the presence of MetS, the HAIR model, acNASH, NAFIC score, Visceral Adiposity Index have also been studied.

Conclusion. A variety of diagnostic panels have shown good predictive values for diagnosing NASH. Nevertheless, non-invasive surrogate markers are currently unable to replace liver biopsy. However, their clinical significance is mainly in triaging patients for liver biopsy, reducing the financial burden associated with the procedure.

Keywords: non-alcoholic steatohepatitis (NASH), surrogate markers, biomarkers, non-invasive, cytokeratin-18 (CK-18), NASH panels, liver biopsy

Introduction

Excessive hepatic fat accumulation in the absence of significant alcohol consumption or other causes of secondary hepatic steatosis is defined as non-alcoholic fatty liver disease (NAFLD) [1-3], a common condition with increased morbidity and mortality as well as several intrahepatic and extrahepatic complications [4-7]. There are currently no approved therapies for the treatment of NAFLD [8-11]. Several risk factors might increase the likelihood of developing NAFLD, including metabolic syndrome (MetS), obesity, diabetes, increased cholesterol levels, sedentary lifestyle, and genetic predisposition [12-14].

Non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH), and cirrhosis are all part of the NAFLD spectrum [2,15]. Unlike NAFL, which typically has a benign clinical course and low mortality rates, NASH has a more progressive course that leads to liver cirrhosis in around 10-15% of patients, possibly leading to liver failure and hepatocellular carcinoma [16]. Furthermore, long-term longitudinal research has shown that NASH is linked to poorer survival rates [16,17]. As a result, identifying NASH is critical for risk

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stratification, limiting disease development, and preventing future consequences.

Given the global prevalence [18], the gold standard test for diagnosing NASH and liver fibrosis, a histological examination of the liver through a biopsy, is a hindrance due to its limitations, such as its invasiveness, which can result in complications such as bleeding, pain, and, in some cases, death, as well as its cost and sampling errors [19]. As a result, liver biopsy is not appropriate for screening in the general population. Because of this, non-invasive diagnostic and staging approaches are constantly being researched. As a result, liver biopsy is rarely used in clinical practice to diagnose and stage NASH. Clinicians commonly integrate noninvasive serum tests, imaging data, and endoscopic discoveries in order to come to a personalized diagnosis and risk stratification for each patient in real life.

It is indeed worth noting that fibrosis development differs widely and is unpredictable among NASH patients [20]. Given that a diagnosis of NASH and fibrosis phases has been closely connected to the risk of clinical outcomes and responsiveness to treatment, the significance of non-invasive diagnostic biomarkers of NASH becomes even more relevant [21]. By providing reliable, quantifiable, and repeatable markers to diagnose and monitor NASH activity and fibrosis staging, as well as to evaluate treatment, noninvasive surrogate markers can enable us to examine risk factors for disease development [22].

Due to the varying rates of disease progression and the large number of circulating biomarkers, there are several issues with the use and accuracy of various accessible surrogate markers in NASH diagnosis. In this review, we summarize several currently evaluated proinflammatory markers, necroinflammation markers, as well as complex clinical and biochemical models which include scores and indexes in assessing NASH.

Proinflammatory markers in NASH

In order to distinguish NASH from simple steatosis, several proinflammatory makers have been examined in NASH. These include C-reactive protein (CRP), ferritin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), pentraxin-3 (PTX-3), and neutrophil extracellular traps (NETs). Conflicting results regarding the ability of high sensitivity CRP (hs-CRP) in differentiating between NASH and hepatic steatosis have been published. According to one study, hs-CRP may be a clinical feature that differentiates NASH from simple non-progressive steatosis and also predicts the severity of hepatic fibrosis in NASH patients [23]. However, another study reported no significant difference between patients with simple fatty liver and those with NASH [24]. Increased serum ferritin levels were seen in NASH patients compared to controls and NASH patients compared to NAFL patients [25]. Despite not presenting a significant association with histopathological findings, serum TNF-α levels were reported to be increased significantly in NASH and liver cirrhosis patients, compared to controls [26]. IL-6 has also been linked to insulin resistance, as evidenced by multiple studies that found a strong association between IL-6 and NASH [27,28]. A study found that NAFLD patients had significantly higher PTX3 levels than controls, and that biopsy-proven NASH patients had elevated PTX3 levels than non-NASH patients [29]. Thus, the authors demonstrated that plasma PTX3 may be a promising biomarker for the existence of NASH in this population. NASH patients had significantly greater levels of NETs markers in their plasma than healthy controls [22]. Furthermore, research has demonstrated that NETs play a key role in developing hypercoagulability in NASH patients.

Necroinflammation biomarkers in NASH

To assess and diagnose NASH, a variety of biomarkers evaluating inflammation and apoptosis including adipocytokines and lipid oxidation panels are studied in the current literature. Studied biomarkers include plasminogen activator inhibitor-1 (PAI-1) and adipocytokines, as well as products of free radical-mediated oxidation of linoleic acid (9- and 13-HODEs and 9- and 13-oxoODEs). In a recent meta-analysis, the authors reported that serum PAI-1 levels were significantly elevated in NASH patients compared to controls [30]. Another systematic review and meta-analysis reports findings suggesting that elevated levels of circulating cytokeratin-18 (CK-18) and fibroblast growth factor 21 (FGF-21) are linked to NASH and can be utilized as a screening tool, but still not enough [31]. A recent study found that leptin increased significantly in NAFLD vs. controls, with similar leptin levels between obese and non-obese healthy controls, suggesting that obesity is not a confounder [32]. Furthermore, adiponectin levels were considerably decreased in NASH compared to NAFLD patients. The assay performance was improved by combining adiponectin with particular serum lipids. With advanced fibrosis, insulin-like growth factor-1 (IGF-1) levels were much reduced, but when combined with the international normalized ratio (INR) and ferritin, the assay performance was significantly improved. Patients with NASH had considerably higher levels of products of free radical-mediated oxidation of linoleic acid (9- and 13-HODEs and 9- and 13-oxoODEs) in their plasma when compared to individuals with simple steatosis and normal liver biopsies [33]. Moreover, the oxNASH, which consists of the Linoleic acid:13-HODE ratio, as well as age, aspartate transaminase (AST), and body mass index (BMI), has been reported to be accurate as a predictive biomarker of NASH and fibrosis staging in several studies [33,34].

Clinical and biochemical models in NASH

Several models have been investigated for their efficacy in predicting NASH using a combination of clinical criteria and biomarkers. An investigated NASH diagnostic

panel that included CK-18-M65, CK-18-M30, resistin, and adiponectin, found that the M65 component had a better predictive value than the M30 component [35]. The NashTest is another panel that includes age, sex, weight, height, triglycerides, cholesterol, α2-macroglobulin, apolipoprotein A1 (ApoA1), AST, alanine transaminase (ALT), haptoglobin, gamma-glutamyl transferase (GGT), and bilirubin [36]. NashTest-2, a quantitative test based on a simplified form of the Clinical Research Network NASH definition, was found to be successful in predicting NASH [37]. Pairing CK-18 fragments with other biomarkers has been shown to improve accuracy in another panel named the NICE model, which combines CK-18 fragments with ALT and the presence of MetS [38]. Elevated ALT levels (>40 U/L) are used in the HAIR model as a NASH predictive panel, among other parameters including hypertension and Insulin Resistance Index >5 [39], whereas a HAIR score of 2 provides a sensitivity of 80% and specificity of 0.89% for predicting NASH, and a score of 3 confirms the presence of NASH [40]. For predicting NASH, a new panel named the acNASH, which combines serum creatinine and AST outperformed prior mentioned panels [41]. The NAFIC score, which includes insulin, ferritin, and type IV collagen 7S is another score with a good predictability for NASH [42]. Another hepatic steatosis score that is linked to insulin resistance is the Visceral Adiposity Index (VAI), that was shown to present an acceptable predictive value for predicting NASH [43].

Conclusions

For diagnosing NASH, a number of diagnostic panels have shown good predictive values. Because many non-invasive surrogate markers are not readily available, accessibility and having cheaper alternatives are important concerns. Non-invasive biomarkers are yet unable to substitute for liver biopsy and histological assessment. They may, however, play a key and vital role in triaging patients for liver biopsy, lowering the related financial burden.

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