Abstract

Background: NAFLD has become a worldwide epidemic. In the US NAFLD is present in 20-40% of the general population, and nonalcoholic steatohepatitis (NASH) is present in about 25% of the obese population. 10-20% of the NAFLD patients develop cirrhosis, and 45-57% of those develop hepatocellular carcinoma. The major risk factors for NAFLD and its cardiovascular phenotype, type 2 diabetes mellitus, dyslipidemia, and metabolic syndrome. NAFLD may be associated with raised blood levels of the liver enzyme aspartate aminotransferase (ALT) and gamma glutamyl transferase (GGT) and associated with increased risk of cardiovascular disease, diabetes, and steatohepatitis. This overexpression and enzyme activity results in excessive, invasive, and toxic to sampling error. Because of these issues repeated biopsies for monitoring are less desirable. A noninvasive method is to detect serum and steatohepatitis to replace liver biopsy and aid in clinical decision-making will represent a significant advancement.

The goal of this study was to develop a serum protein signature for:
1. Detect and monitor severity of steatohepatitis
2. Detect and monitor severity of steatohepatitis

Methods: Serum samples were from a retrospective study where liver biopsies were performed on 546 obese patients who underwent bariatric surgery for weight loss at the Geisinger Clinic. Of these, 150 had normal biopsies; 182 showed varying degrees of steatosis (mild, moderate and severe); and 213 showed various stages of NASH (1-3). Biomarker discovery was performed using SomaLogic’s highly multiplexed SOMAmer™ proteomics array, which measured 1,152 proteins simultaneously from 45µL serum. Demographics such as age, BMI and LAB, cholesterol levels were balanced across all groups. Candidate markers were selected using a stability selection algorithm with a L1-regularized logistic regression kernel.

Results: Two random forest classifiers were developed. The first, which was designed to detect liver steatosis, compared controls versus all NAFLD groups and added an area under the ROC curve (AUC) of 0.898±0.012 (95% CI). The second, which was designed to predict NASH, compared controls versus all NASH groups and added an area under the ROC curve (AUC) of 0.856±0.010 (95% CI). Both NAFLD classifiers had elevated probabilities of steatohepatitis classifier score. The top 10 proteins (from total of 1520) had a Benjamini corrected value of 1.234E-4 and a KS-distance of 0.349.

The second consisted only groups with hepatic steatosis and distinguished NASH (stage 1-3) from liver without NASH (mild and moderate steatosis). This hepatitis NAFLD classifier had an AUC of 0.864±0.021 (95% CI). The probability score of NASH classifier showed the highest variation with severity of steatosis.

Conclusions: In this initial study we have discovered a set of serum protein biomarkers that are highly associated with liver steatosis and NASH. This result is associated with metabolism, matrix remodel, apoptosis, and inflammation. These results will be further validated on blinded and a larger sample set.

Results: NASH Classifier

A nine marker classifier to detect NASH fibrosis in obese individuals

Table: NASH Classifier

- Using the SOMAmer proteomic technology we have discovered serum protein biomarkers of NAFLD
- A nine marker classifier to detect NASH fibrosis in obese individuals
- The markers were associated with metabolism, matrix remodeling, apoptosis, and inflammation.
- The performance of the models will be evaluated on the 25% blinded sets
- These results will be further validated on a larger sample set –NASH.CRN

Potential Clinical Utility: A noninvasive blood test to identify in obese individuals, those with liver steatosis and/or steatohepatitis to aid in a lifestyle change regime or treatment decision

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