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Predicting Fibrotic NASH, or At-Risk NASH, with the SomaScan® Assay

Introduction

Non-alcoholic steatohepatitis (NASH) is a combination of liver inflammation, cell destruction, and accumulation of fat and scar tissue in the liver. NASH can progress to advanced liver fibrosis and cirrhosis, leading to liver failure. The rapidly growing incidence of NASH worldwide is linked to the increase in type 2 diabetes and obesity, and it has become the most common chronic liver disease.

Liver biopsy and histology remains the established reference standard for diagnosis of NASH. However, this method is invasive, resource intensive, prone to sampling and inter-observer errors, and carries a small but significant risk of complications. Consequently, there is an urgent need for the development of biomarkers in NASH as a noninvasive replacement for liver biopsy for use in diagnosis and monitoring of disease progression and treatment response.

Clinical trial enrollment for NASH drug trials typically requires participants with NASH and significant fibrosis (fibrosis stage of at least 2).[1] This condition has been termed fibrotic NASH, or "at-risk NASH." Patients with fibrotic NASH are at higher risk for disease progression and are ideal candidates for response to NASH therapies that target liver inflammation and fibrosis.

Therefore, a noninvasive blood test that can predict the likelihood that an individual has fibrotic NASH could accelerate drug development by increasing efficiency of trial enrollment and response monitoring.

Currently, validated serum-based SomaSignal™ tests to independently predict the 4 components of liver biopsy that characterize NASH and fibrosis (steatosis, inflammation, ballooning and fibrosis) are available for research use. The four NASH biopsy component models (steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis) are elastic net logistic regression models containing 12, 14, 5, and 8 features, respectively. The classes used to develop the model (the "truth standards") are based on the NAS histological scoring system for Nonalcoholic Fatty Liver Disease.[5] Output of each model is a predicted probability, captured in the test report as "Predicted Value," and is a continuous score ranging from 0 to 1. The predicted probability is then used to divide the component into two classes based on a specified decision threshold of 0.5, with higher probabilities indicating a higher likelihood the individual has more advanced liver pathology. See Table 1 for predicted probabilities as they relate to the predicted class for each model, and the binary histological decision thresholds used for model development.

Liver Biopsy Component	Product ID	Component Test Name	Predicted Prob <0.5 (Negative Predicted Class)	Predicted Prob ≥0.5 (Positive Predicted Class)
Steatosis	R1112	NASH Steatosis	Steatosis Score 0	Steatosis Score 1, 2 or 3
Lobular Inflammation	R1113	NASH Inflammation	Inflammation Score 0 or 1	Inflammation Score 2 or 3
Hepatocellular Ballooning	R1114	NASH Ballooning	Ballooning Score 0	Ballooning Score 1 or 2
Fibrosis	R1115	NASH Fibrosis	Fibrosis Stage 0, 1a, 1b, or 1c	Fibrosis Stage 2, 3 or 4

TABLE 1 Definition of binary histologic classes and translation to NASH Test Reports

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SL: 00000669 Rev 1 - 2021-09
Predicting Fibrotic NASH

A positive result for each component (predicted probability > 0.5) corresponds to the description of fibrotic NASH:

- NAS score of at least 4, with at least 1 in all 3 NASH components (steatosis, inflammation and ballooning)
- Plus fibrosis stage of at least 2.

This technical note describes the validation results from combining the existing models to predict this combined endpoint, fibrotic NASH. Fibrotic NASH can be predicted simply by multiplying the four SomaSignal™ test predicted probability outputs.

SomaSignal NASH tests

The four SomaSignal NASH tests noninvasively predict the histological components of liver biopsy (steatosis, inflammation, hepatocellular ballooning, fibrosis) in individuals with suspicion of NASH using algorithms based on quantitative protein patterns. The tests were developed and validated with serum samples from the NASH Clinical Research Network, including participants from a natural history cohort and longitudinal samples from the PIVENS[3] (pioglitazone, vitamin E and placebo) and the FLINT[4] (obeticholic acid and placebo) clinical trials. Liver biopsy results were modeled with measured proteins using machine learning methods independently for each biopsy component (see SomaSignal NASH Test Information Guide for detailed study design and validation results[2]). Results for the 4 protein models in training/validation were: fibrosis (AUC 0.92/0.85); steatosis (AUC 0.95/0.79), inflammation (AUC 0.83/0.72), and ballooning (AUC 0.87/0.83) (Figure 1).

In an exploratory analysis to identify individuals with cirrhosis (fibrosis stage 4), preliminary data using a different threshold for the probability output of the fibrosis model yielded promising results. Using a 0.95 probability cutoff in the fibrosis model allowed identification of 17 out of 25 individuals with cirrhosis in the training set, and 2 of 3 and 7 of 10 in the two validation sets respectively. The overall specificity for diagnosis of cirrhosis was 86%.

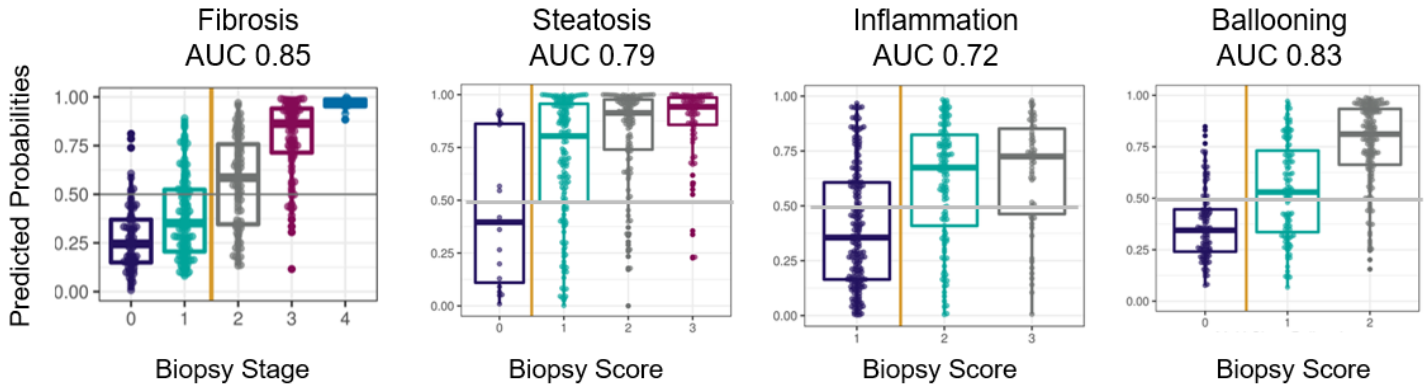

FIGURE 1 Validation performance for each NASH component SomaSignal test compared to liver biopsy. Yellow vertical line indicates the binary class threshold and gray horizontal line indicates the model decision threshold of 0.50. Biopsy stage or score is based on the non-alcoholic fatty liver disease activity score (NAS).[5]

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SL: 00000669 Rev 1 - 2021-09
Predicting Fibrotic NASH

Combining SomaSignal™ NASH test results to predict Fibrotic NASH

A common clinical trial entry requirement, based on FDA guidance,[1] is for the presence of fibrotic NASH, or "at-risk NASH", defined as a NAS of at least 4 and fibrosis stage of at least 2. These individuals are at high risk for disease progression. This composite endpoint can be predicted with a combination of the output from each of the four individual liver biopsy component SomaSignal tests.

Fibrotic NASH can be predicted by multiplying the four SomaSignal test predicted probability outputs. The threshold for binary classification of fibrotic NASH is the product of the 4 model decision thresholds, or 0.0625 (0.5^4), where > 0.0625 is classified as positive for fibrotic NASH. The performance of this composite model, applied to the same validation data set as the individual component tests, resulted in an AUC of 0.85 and sensitivity/specificity/accuracy of 87%/63%/73% in validation (Figure 2).

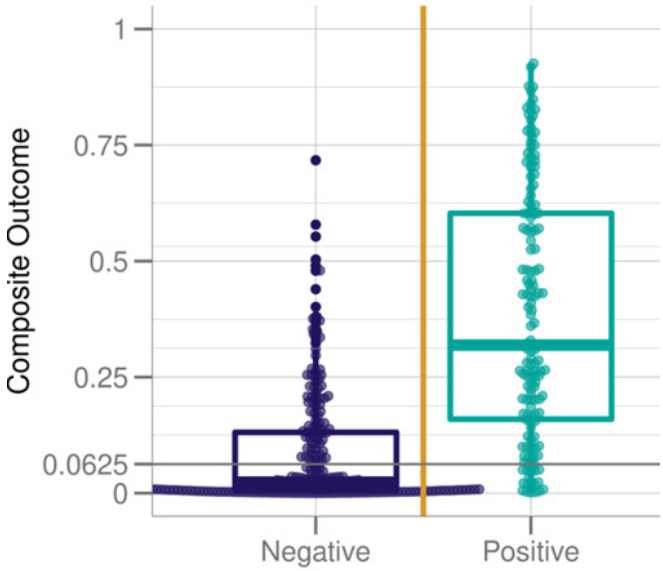

FIGURE 2 Validation performance for prediction of fibrotic NASH. Yellow vertical line indicates the binary class threshold and gray horizontal line indicates the model decision threshold of 0.0625.

Independent Validation of NASH SomaSignal tests

Independent validation of the same proteomic models described here has been demonstrated by the LITMUS investigators in a collection of samples from individuals with biopsy-proven NAFLD (n=267, 59% with histological evidence of NASH, 59% with significant fibrosis).[6,7] The AUC for detection of biopsy-confirmed NASH was 0.78 (95% CI 0.73-0.84) for the proteomic models, which was significantly better than FIB-4, which had an AUC of 0.61 (95% CI 0.55-0.68). In addition, the AUC for detection of significant fibrosis was 0.86 (95% CI 0.81-0.90), compared to FIB-4 AUC of 0.67 (0.60-0.73). The authors further conclude that "the SomaScan proteomic algorithm could substantially reduce histological screen failure rates when biopsy was reserved for only biomarker positive patients" if used to identify at-risk NASH patients for therapeutic trial recruitment.[8]

Conclusion

The NASH SomaSignal tests are novel non-invasive tests to predict the four individual components of liver biopsy. When multiplied together, they predict the presence of fibrotic NASH (NAS of at least 4 and fibrosis stage of at least 2), which is the condition commonly required for NASH drug trial enrollment. The additional benefit of predicting fibrotic NASH along with the individual SomaSignal tests from a single serum sample provides a complete non-invasive combination of test results which could accelerate drug development programs.

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SL: 00000669 Rev 1 - 2021-09
Predicting Fibrotic NASH

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