***Title***

**Sequential combination of NIS4, MACK-3, and FAST scores: toward a more accurate non-invasive algorithm to identify ‘at-risk’ NASH**

**Authors:**

Yu-Jie Zhou1,2, Kenneth I. Zheng1, Giovanni Targher3, Christopher D. Byrne4, and Ming-Hua Zheng1,5,6\*

**Institutions:**

1 NAFLD Research Center, Department of Hepatology, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China;

2 Division of Gastroenterology and Hepatology, Key Laboratory of Gastroenterology and Hepatology, Ministry of Health, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai Institute of Digestive Disease, Shanghai, China;

3 Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy;

4 Southampton National Institute for Health Research Biomedical Research Centre, University Hospital Southampton, Southampton General Hospital, Southampton, UK;

5 Institute of Hepatology, Wenzhou Medical University, Wenzhou, China;

6 Key Laboratory of Diagnosis and Treatment for The Development of Chronic Liver Disease in Zhejiang Province, Wenzhou, China.

**\*Corresponding Author:**

Ming-Hua Zheng, MD, PhD

NAFLD Research Center, Department of Hepatology, the First Affiliated Hospital of Wenzhou Medical University; No. 2 Fuxue Lane, Wenzhou 325000, China.

E-mail: zhengmh@wmu.edu.cn; fax: (86) 577-55578522; tel: (86) 577-55579622.

**Electronic word count:** 495 + n=5 references

There is an urgent need to identify patients with ‘at-risk’ nonalcoholic steatohepatitis (NASH), i.e. defined as NAFLD activity score ≥4 and fibrosis stage ≥2 on liver histology, as these patients are required to test the efficacy of new pharmaceutical drugs for NASH in clinical trials. Traditional non-invasive tests (NITs) have been developed to identify either NASH or advanced fibrosis, such as cytokeratin-18 (CK-18) fragments M30/M65, Enhanced Liver Fibrosis (ELF), fibrosis (FIB)-4 and NAFLD fibrosis score. However, these NITs do not perform well in detecting ‘at-risk’ NASH. Recently, Harrison *et al*.1 developed and validated a novel blood-based diagnostic test (namely the NIS4 algorithm) that provides an effective way to rule in or rule out ‘at-risk’ NASH (with an area under the receiver operating characteristics curve of 0.80, 95% CI 0.73-0.85).

To our knowledge, there are only two other NITs that can be used to detect ‘at-risk’ NASH, i.e. the Fibroscan-AST (FAST) and MACK-3 scores.2, 3 Specifically, the NIS4 (that includes miR-34a-5p, alpha-2 macroglobulin, YKL-40, and glycated hemoglobin in its equation), and the MACK-3 score (that includes homeostasis model assessment-estimated insulin resistance, serum AST and CK-18 levels) are two blood-based diagnostic tests, while the FAST score (that includes liver stiffness measurement, controlled attenuation parameter, and serum AST levels) is principally derived from Fibroscan®.

All these NITs usually provide two cutoff points: one with the highest sensitivity and one with the highest specificity. Consequently, between these two cutoff points there is a ‘grey zone’ of indeterminate results, and for the FAST and MACK-3 scores, nearly 30% of test scores are in that ‘grey zone’. For example, for the NIS4 score, with 0.36 and 0.63 as lower and higher cutoff points, ~30% of patients in the training cohort and 27% of those in the validation cohort, respectively, fell into the ‘grey zone’.1 Importantly, in the validation cohort, 56% of the patients in the ‘grey zone’ had ‘at-risk’ NASH confirmed by liver histology.1 Thus, it is essential to find a more accurate test to identify patients with ‘at-risk’ NASH, who are in the ‘grey zone’ of NIS4.

The sequential combination of NITs designed for liver fibrosis detection accurately classifies more than 90% of patients with advanced NAFLD fibrosis.4 In a similar way, we propose that the sequential combination of NIS-4, MACK-3 and FAST scores might help to narrow the ‘grey zone’ and further avoid unnecessary liver biopsies in ‘grey-zone’ patients. It has been shown that the FAST score relies heavily on serum AST levels and thus it accurately classifies cases with significant fibrosis, but may also place cases with normal serum liver enzymes in the ‘grey zone’.5 In these cases, we propose that additional testing with NIS4 or MACK-3 might better identify ‘at risk’ NASH, since these two blood-based diagnostic tests include in their equations other important parameters associated with ‘at risk’ NASH (CK-18, YKL-40, insulin resistance and glycated hemoglobin). We suggest that our proposal to combine sequentially these three newly developed NITs to better identify ‘at-risk’ NASH warrants further research.

**References**

1. Harrison SA, Ratziu V, Boursier J, et al. A blood-based biomarker panel (NIS4) for non-invasive diagnosis of non-alcoholic steatohepatitis and liver fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol* 2020.

2. Newsome PN, Sasso M, Deeks JJ, et al. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol* 2020;**5**:362-73.

3. Boursier J, Anty R, Vonghia L, et al. Screening for therapeutic trials and treatment indication in clinical practice: MACK-3, a new blood test for the diagnosis of fibrotic NASH. *Alimentary pharmacology & therapeutics* 2018;**47**:1387-96.

4. Boursier J, Guillaume M, Leroy V, et al. New sequential combinations of non-invasive fibrosis tests provide an accurate diagnosis of advanced fibrosis in NAFLD. *Journal of hepatology* 2019;**71**:389-96.

5. Noureddin N, Alkhouri N, Brown KA, Noureddin M. Driving NASH forward using the FAST score but obey the traffic lights. *Hepatology* 2020.