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Editorial

Predictive models with the use of omics and supervised machine learning to diagnose non-alcoholic fatty liver disease: A "non-invasive alternative" to liver biopsy?

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Non-alcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease worldwide, affecting 25–30% of the general population [1], with its prevalence reaching 55% in patients with type 2 diabetes mellitus (T2DM) [2] or even 90% in morbidly obese individuals [3]. NAFLD is characterized by fat accumulation in ≥5% of the hepatocytes, in the absence of excessive alcohol consumption or other secondary causes of liver diseases [4,5]. At the early stages of NAFLD, there is only liver steatosis (i.e. NAFL), which can then progress to liver inflammation and fibrosis (i.e. NASH), and, potentially, to cirrhosis and hepatocellular carcinoma (HCC) [4,5]. Importantly, NAFLD has been linked to increased liver, T2DM and cardiovascular (CV) morbidity and mortality [6–9]. NAFLD is now recognized as an important health problem with an urgent need for early diagnosis.

NAFLD is a metabolic disease, thus the use of lipidomic and metabolomic techniques might provide useful diagnostic biomarkers. In this issue of *Metabolism*, Perakakis et al. [10] propose non-invasive models consisting of lipids, glycans and hormones that can simultaneously diagnose the presence of NAFL, NASH or healthy status with very high accuracy (>90%). Furthermore, they report a combination of lipids that can diagnose liver fibrosis with very high accuracy (98%) [10]. Briefly, a stepwise lipidomic, glycomic and free fatty acid analysis, as well as multiple machine learning methods, were performed in 80 participants: 49 healthy individuals and 31 patients with biopsy-proven with NAFL ($n = 15$) or NASH ($n = 16$) [10].

After excluding the lean healthy participants, 32 lipid species could discriminate obese healthy vs NAFL vs NASH patients, with similar representation (i.e. phosphatidylethanolamines, phosphatidylcholines and sphingomyelins), thus suggesting body mass index (BMI)-independent differences in lipidome between these groups [10]. Furthermore, significant differences between groups were observed for 17 glycans; only 5 glycans remained significantly different after excluding the lean controls [10]. Among them, only 2 glycans could partially discriminate the healthy from the NAFL and NASH groups (both before and after excluding the lean healthy participants) but could not distinguish NAFL from NASH patients. Regarding free fatty acids (FFAs), 5 of them could partially discriminate healthy vs NAFL vs NASH patients, independently of BMI [10].

Following these analyses, the authors evaluated the predictive accuracy of NASH, NAFL or healthy status via 5 machine learning methods. Overall, a very high predictive accuracy for all the 3 groups was achieved with the combination of 29 different lipid species, whereas hormones and FFAs demonstrated a high accuracy for discriminating NASH and healthy status (but low for predicting NAFL). In contrast, glycans were able to better predict NAFL compared with hormones and FFAs, but were worse for discriminating NASH and healthy status.

Furthermore, 21 NAFLD patients (out of 31) had liver fibrosis (diagnosed by liver biopsy). Lipidomics (consisting of 10 lipid species) were able to differentiate robustly (with 99% specificity and 95% sensitivity) the presence of liver fibrosis in these patients, whereas FFAs and glycans were less predictive [10]. These diagnostic models should be further tested and validated prospectively in larger and more heterogeneous populations.

This pilot case-control study [10] highlights the beginning of a new era in the diagnosis and treatment of NAFLD using non-invasive tests based on omics and supervised learning. If well-validated in different populations, these models may serve as a useful, non-invasive, cost-effective, low-risk, method for diagnosing and staging NAFLD, thus representing an attractive alternative to liver biopsy that is currently the gold-standard for diagnosing and staging NAFLD [4,11,12]. Taking into consideration the increasing worldwide prevalence of NAFLD and its multisystemic nature, linked to increased morbidity and mortality, as well as the significant limitations of performing liver biopsies, there is an urgent need for reliable, accurate, non-invasive tests to predict NAFLD. Regarding imaging procedures, liver ultrasound (US) has many advantages (e.g. simple, non-invasive, relatively inexpensive, easy to perform and well tolerated) and it is widely used for the diagnosis of NAFLD [4,5,13], However, hepatic US also has certain disadvantages; e.g. it is qualitative and thus subjective, its sensitivity and specificity decrease with increasing BMI; none of the imaging techniques can differentiate NAFL from NASH [14].

Other techniques to measure liver stiffness and assess the presence of fibrosis include the vibration controlled transient elastography (VCTE) or FibroScan, magnetic resonance elastography (MRE) and acoustic radiation force impulse techniques [13,15,16]. However, these techniques have certain limitations (e.g. increased cost, time consuming, technical difficulties, restricted availability, no widely validated cut off values, limited reliability especially in severely obese patients), that minimize their clinical value in the daily practice. Newer imaging techniques, e.g. magnetic resonance spectroscopy (MRS), MRI-proton density fat fraction (PDFF) measurements and positron emission tomography (PET), still have certain limitations and their accuracy and

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reliability in diagnosing NAFLD remain to be established in clinical practice [13,14]. Radiomics and radiogenomic studies may also provide a new field for NAFLD diagnosis but considerable research is needed to reach acceptable intra- and inter-reader variations of this digital biopsy.

In terms of non-radiological diagnostic tests, there are several scores/indexes combining clinical variables and/or serum biomarkers to predict the presence of fibrosis or NASH [15]. Such tests range from the simplest predictive model for fibrosis, i.e. the aspartate aminotransferase (AST)-to-alanine aminotransferase (ALT) ratio, to more complex scores, including the frequently used BARD score (using BMI, AST-to-ALT ratio and diabetes), the FIB4 index (combing age, AST, ALT and platelet count), the fatty liver index (FLI) [using BMI, waist circumference, triglyceride and gamma-glutamyltransferase (γGT)], the NAFLD fibrosis score (NFS) (including BMI, age, albumin, AST-to-ALT ratio, platelet count and impaired fasting glucose/diabetes) and the FibroTest (using α2-macroglobulin, haptoglobin, total bilirubin, apolipoprotein A1 and γGT) [4,5,15,16]. Novel diagnostic approaches for NASH involve pro-inflammatory cytokines such as interleukin 6 and tumor necrosis factor α (TNFα), the intermediate filament protein cytokeratin 18 (CK 18), microparticles, gut microbiota and volatile organic compounds (VOCs) in exhaled breath, as well as the enhanced liver fibrosis (ELF) test (including tissue inhibitor of metalloproteinase 1, hyaluronic acid and aminoterminal peptide of procollagen III) [4,5,16]. Homeostasis model assessment of insulin resistance (HOMA-IR) cut-off values for NAFLD diagnosis have also been suggested [17]. Of note, genetic predisposition for NAFLD has been reported [18,19]; a single nucleotide polymorphism (SNP) in the patatin-like phospholipase domain-containing protein 3 (PNPLA3) (i.e. rs738409) on chromosome 22 was strongly related to increased hepatic inflammation and fat levels [18,19]. In this context, the combination of genetic and metabolic parameters have been proposed to improve the score to diagnose NAFLD [20] and an extended FLI was calculated based on the oral glucose tolerance test (OGTT)-derived fold-change in plasma triglycerides along with 2-h blood glucose and the rs738409 C>G SNP in PNPLA3, showing improved predictive power for NAFLD diagnosis [21].

However, most of the above mentioned indexes have been derived using data of patients that underwent liver biopsy and thus often had advanced liver disease. Moreover, there are no validated scores to identify patients with NASH; thus, NASH remains undiagnosed if a liver biopsy is not performed. Furthermore, NAFLD is a metabolic disease and its pathophysiology involves lipid metabolism, adipose tissue, liver, gut and pancreatic hormones [22,23]. In this context, the development of a predictive model using omics (lipidomics, glycomics), FFAs and hormones, as well as supervised learning machines, as proposed by Perakakis et al. [10], may help differentiate NAFL, NASH and healthy individuals, as well as diagnose liver fibrosis with very high accuracy.

This targeted lipidomic analysis may be more cost-effective than liver biopsy; as mentioned by the authors [10], the cost of liver biopsy may vary between $2000–8000 in the USA. In contrast, the costs of the model suggested by Perakakis et al. [10], combining lipids, adiponectin and glycans, is $605/individual, thus significantly lower than that of liver biopsy and the cost is expected to be further decreased as mass-spectrometric methods become more available and targeted measurements of lipids and glycans are performed [10]. Moreover, target lipidomic analysis gives information also on the metabolic status and may be easily repeated over time, being non-invasive, allowing to monitor improvement/regression of the disease.

Liver biopsy has certain limitations, including potential complications (e.g. pain, hemorrhage, infection and need for hospitalization) and the majority of the patients may be reluctant to undergo this invasive diagnostic procedure, thus minimizing its clinical relevance. Therefore, many cases of NAFL may remain undiagnosed and untreated. It follows that an accurate and easy to perform diagnostic method for NAFL based on targeted omic analysis (e.g. as described by Perakakis et al. [10]) may overcome these limitations of liver biopsy and help diagnose NAFL in all patients, thus avoiding disease progression and the development of CV and liver complications. Such an approach appears to be cost-effective. Furthermore, the availability of such a predictive model may facilitate drug therapy research for NAFL, as more patients will be able to participate in trials.

Overall, the diagnostic model developed by Perakakis et al. [10] should be further validated in different populations, to establish whether it indeed represents a reliable and accurate, "non-invasive alternative" to liver biopsy. The clinical implications of such findings will be significant, highlighting the initiation of a new era in the diagnosis, staging and treatment of NAFLD.

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