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POTENTIAL ROLE OF A PREDICTIVE BIOMARKER IN THE PROGRESSION OF FATTY LIVER DISEASE

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in industrialized countries, affecting about 25.24% of the general population. NAFLD is a benign disease, but it can develop into more serious diseases, including non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and finally hepatocellular carcinoma (HCC). Liver biopsy is still the gold standard for diagnosing NAFLD. Due to the risks associated with liver biopsy and the inability to apply it on a large scale, it is now necessary to identify non-invasive biomarkers that can reliably identify patients at increased risk of progression. Thus, in this review, we report on biomarkers used in clinical practice for diagnosing NASH as well as for staging fibrosis, and secondly, we draw attention to new biomarkers, miRNAs, long non-coding RNAs, for diagnosing and staging fibrosis. This review discusses the potential role of miRNAs in the pathogenesis of fatty liver disease.

Key words: biomarkers; miRNA; NAFLD, intestines, triglyceride, glycemia, fibrosis.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in industrialized countries, and this is associated with an increase in morbidity and mortality. NAFLD is a hepatic manifestation of the metabolic syndrome, which is

considered systemic disease, as it affects various organs, including the pancreas [1], kidneys, adipose tissue and intestines [2,4]. It is estimated that NAFLD affects about 25% of the total population [5], although the actual prevalence is unknown due to the lack of specific and sensitive diagnostic tests. NAFLD includes several pathological conditions ranging from from simple steatosis to non-alcoholic steatohepatitis (NASH), the latter characterized by non-hemorrhagic inflammation of the liver and rapid progression towards fibrosis, cirrhosis and, finally, hepatocellular carcinoma (HCC) [5,8]. Liver biopsy is still the gold standard for differentiating simple steatosis from NASH and stages of fibrosis. Over the past few years, the identification of non-invasive biomarkers Differentiating simple steatosis from NASH and stages of fibrosis has become one of the most prevalent research objectives in the field of hepatology. This question is very important because the high prevalence of NAFLD in the general population, the potential for progression to more serious disease, and the inability to use liver biopsy on a large scale dictate large-scale studies.

Biomarkers. The diagnosis of NAFLD includes the definition of hepatic steatosis (defined as the histological presence of triglyceride accumulation in 5% of hepatocytes) as an exception there was a selection among those examined with liver damage not taking or taking a limited amount alcohol <20/30 g per day for women and men, respectively [9,10]. According to the literature, several indices and scales for the diagnosis of hepatic steatosis have been developed over the past decades [13,14]. FLI (fatty liver index) includes BMI, waist circumference, serum triglycerides and GGT (gamma-glutamyl transferase). FLI has a moderate diagnostic efficiency (AUC = 0.84), it has a low accuracy in determining several degrees of steatosis [16]. The LSI (Liver Steatosis Index) is a panel of biomarkers that includes ALT/AST, BMI, gender, and the presence of type 2 diabetes, and this index has a moderate diagnostic detectability for hepatic steatosis [17], which is significantly reduced in children with obesity (AUC = 0.67) [18]. In addition, like FLI, it has low accuracy in determining several degrees of hepatic steatosis [16]. A more efficient and sensitive method is the assessment of liver fat in NAFLD; by means of magnetic resonance, this method detects abnormalities in the liver. This indicator has a good diagnostic efficiency for diagnosing liver steatosis [19]. SteatoTest is a panel that includes more specific parameters for diagnosing steatosis. It combines the six elements of FibroTest (α2-macroglobulin, haptoglobin, apolipoprotein A1, GGT, total bilirubin and ALT) in addition to BMI, cholesterol, triglycerides and glycemia. The SteatoTest is adjusted for sex and age but has moderate accuracy for predicting hepatic steatosis confirmed by liver

biopsy (AUC = 0.80) [20]. This test is not widely used because it does not distinguish between different degrees of steatosis.

Visual biomarkers. Imaging techniques are often used in clinical practice and are used to diagnose NAFLD. To date, elastography has a high diagnostic accuracy in distinguishing between moderate and severe steatosis [21]. However, this method has some limitations: unable to detect steatosis when it is less than 20%, and depends on the skill of the operator [11]. Magnetic proton density resonance imaging of the fat fraction (MRI) is a non-invasive method that displays fatty degeneration of the liver. MRI is more accurate in detecting multiple degrees of steatosis in patients with NAFLD [23]. Based on the foregoing focus on biochemical and molecular biomarkers involved in specific pathways of progression of pathological process is the main point in the prognosis and diagnosis of various complications of NAFLD. Scientists from Harvard University in the nematode C. e legance [1] discovered the first miRNA, named lin-4, a quarter of a century ago. Scientists found that the lin-4 gene did not encode a protein, but two small RNAs - a precursor of 61 nucleotides in length and micro-RNA itself, of 22 nucleotides, which suppressed the expression of the lin-14 nematode gene, preventing it from developing normally. For a long time it was believed that microRNA is such an evolutionary exotic, a property of the nematode genome, until seven years later, in 2000, the second miRNA molecule, let-7, was discovered [2]. It suppressed the expression of several genes at once and was then described in a number of living organisms, including humans. And after that, the "dam broke" – microRNA discoveries began to follow one after another[15].

Circulating RNA to determine the stage of fibrosis. Fibrosis associated with NASH includes various stages, from no fibrosis (F0) to liver cirrhosis (F4). From a clinical perspective, fibrosis is defined as clinically significant (F2–F4) or severe/severe fibrosis (F3–F4). It is widely known that there are several risk factors that can predict the onset of fibrosis, including age, severe obesity, type 2 diabetes mellitus, high AST/ALT ratio, hypertension, dyslipidemia, and the presence of metabolic syndrome [31,34]. The stage of liver fibrosis may be the most important factor in determining the prognosis of NAFLD and preventing the risk of progression to cirrhosis and its associated complications [35]. Biological fluids contain RNA molecules belonging to different classes, including messenger RNAs (mRNAs), long non-coding RNAs (lncRNAs), circular RNAs (cirRNAs), and microRNAs. Circulating miRNAs are extremely stable because they are not present in the circulation in free form, they are encapsulated in membrane vesicles, alternatively complex with RNA-binding proteins, or associated with lipoproteins. All these mechanisms protect RNA from degradation [37]. Another aspect that

makes RNA molecules optimal biomarkers is that the methods used to detect them are extremely sensitive. Unlike proteins, nucleic acids are detected and quantified using PCR-based methods. Other studies have reported that differential expression profiles of circulating RNAs correlate with various physiological and pathological conditions [38]. MicroRNAs are widely known to be involved in the pathogenesis of NAFLD, and their dysregulation has been described in NASH-associated fibrosis. Although there are many studies reporting the role of microRNAs in pathogenesis, very few studies have evaluated circulating microRNAs as biomarkers of fibrosis in NAFLD populations. MicroRNA-122 expression was analyzed in 67 NAFLD patients with multiple degrees of fibrosis. MicroRNA-122 levels were significantly lower in patients with severe fibrosis compared with mild fibrosis. The diagnostic performance of miRNA-122 was also evaluated and compared with other fibrosis biomarkers such as hyaluronic acid and type IV collagen. MicroRNA-122 had a higher diagnostic accuracy compared to hyaluronic acid and type IV collagen. These studies support the use of RNA molecules as non-invasive biomarkers. Akuta et al. analyzed miRNA-122 expression levels in patients with NAFLD. They observed that in patients with improved histopathological parameters, serum levels of miRNA-122 expression were reduced at the second biopsy compared to the first biopsy. In addition, there was a significant correlation between miRNA-122 expression levels and histopathological score variations. The authors also observed a relationship between miRNA-122 expression levels and common clinical parameters such as AST and ALT [39]. The study reported that miRNA-21 levels were also reduced in patients with NAFLD compared with the control group [40]. Yamada et al. analyzed the expression of 5 miRNAs involved in lipid homeostasis (miRNAs -21, -34a, -122, -145, -451) in a total of 403 subjects. Serum levels of four of these miRNAs were higher in subjects with NAFLD compared to controls (92/403) (miRNA-21, -34a, -122, -451); in addition, miRNA-122 expression levels correlated with the degree of steatosis [16]. Cermelli et al. in 2013, reported in more detail the increase in miRNA-122, -16 and -34a expression levels in 34 NAFLD patients compared to 19 controls. On average, miRNA-122 showed a 7.2-fold increase in changes, miRNA-34a showed a 5.3-fold increase in changes, and miRNA-16 was not detected in control samples, while pathological samples had 1000 copies per ml. MicroRNA-122 and -34a increased further in patients with NASH compared with those with simple steatosis, with a fold change of about two and three times, respectively. Another important study by Pirola et al. in 2015 was aimed at determining biomarkers for the differential diagnosis of NAFLD and NASH. They profiled the expression of 84 miRNAs in patients with NAFLD,

NASH, and controls (n = 48) and consistently confirmed activation (FC > 2) of a panel of six miRNAs in an independent cohort (n = 96): miR -122, -192, -19a , -19b, -125 and -375. Only three of them, miRNA-122, -192 and -375, correlated with histological severity and were significantly overexpressed in patients with NASH compared to those with NAFLD. miRNA-122 and -192 were further confirmed by Becker et al. as indicators of histological severity. In more detail, they analyzed the expression of four microRNAs, i.e. miRNA-122, -192, -21 and -223, in 137 NAFLD patients with moderate or severe obesity (87 NASH and 50 with simple steatosis (PS) compared to 61 patients. In addition, they performed a correlation analysis between miRNA expression level and common biomarkers such as AST, ALT. Among the miRNAs analyzed, miRNA-122 and miRNA-192 were increased in severely obese NASH patients compared to severely obese CVD patients.

Conclusion. Today, NAFLD poses a global health risk due to its high prevalence in the general population. We know from multicenter studies that the majority of patients with NASH are at high risk of developing liver cirrhosis [29,30]. Therefore it is extremely important early identification of patients with NASH The ability to correctly identify patients with NASH among patients with NAFLD and determine the stage of fibrosis is an important clinical challenge. Currently, liver biopsy is still the gold standard for diagnosing NASH and the stage of fibrosis. However, because of the risks associated with biopsy, biochemical and imaging tests are often used by clinicians, even if they have limited diagnostic performance [13,14,21]. The introduction of new molecular genetic technologies, in particular miRNAs, will make it possible to identify patients with NAFLD in order to determine the stages of fibrosis for the subsequent prognosis of complications.

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