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Mass Spectrometry-Based Proteomics in Alcoholic and Non-Alcoholic Steatohepatitis

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[&]Author Contribution:

Hetland LE, Kimer N, Gluud L and these authors are contributed equally to this work.

1. Abstract

1.1. Aims: This review aims to provide an overview of candidate biomarkers derived from MS-based proteomics representing inflammation or fibrosis in NAFLD and ALD. The prevalence of non-alcoholic fatty liver disease (NAFLD) and Alcoholic Liver Disease (ALD) is increasing worldwide and the associated morbidity and mortality is considerable. Both conditions are characterized by increasing steatosis, inflammation, and fibrosis. Accurate, non-invasive biomarkers are needed to ensure earlier diagnosis and intervention. Mass-spectrometry (MS)-based proteomics is a leading technology within biomarker identification.

1.2. Methods: We identified nine papers describing relevant MS-based analyses of NAFLD and ALD. Results: The studies described potential biomarkers including serum Paraoxonase-1 (PON1), clusterin, lumican and Polymeric Immunoglobulin Receptor (PIGR). The combined evidence suggested that the concentration of PON1, which is an enzyme with anti-inflammatory and anti-atherogenic properties, was lower in patients with NAFLD of all stages, which correlates with previous research on NAFLD and other metabolic diseases. PIGR was upregulated in plasma in ALD and NAFLD but downregulated in the liver in NAFLD. The protein plays a role in mucosal immunity, but its association to NAFLD is not well known. Lumican is a proteoglycan associated with the formation of fibrosis. It was upregulated in plasma from patients with NASH and advanced fibrosis as well as patients with alcoholic hepatitis. Clusterin has protective anti-fibrotic and anti-steatotic effects and was upregulated in liver and plasma from animal models with NAFLD and ALD fibrosis.

1.3. Conclusion: There may be proteins common to the inflammation or fibrotic pathway between the two diseases alcoholic and non-alcoholic steatohepatitis.

2. Introduction

Non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) are increasing in prevalence worldwide [1, 2]. Both conditions can eventually lead to cirrhosis, which is the clinicopathological presentation of end-stage liver characterized by massive scarring of the liver [3]. Cirrhosis is generally preceded by steatohepatitis with fibrosis in a progressive manner (Figure 1).

The number of NAFLD cases is predicted to increase with 63% from 2015 to 2030, resulting in 27 million cases only in the US [2]. In Europe, an expected increase of the NASH subgroup from 2016-2030 was reported as 43% in Germany and 49% in Spain, based on a dynamic Markov model from data from adult prevalence of obesity and Type II Diabetes Mellitus [4]. Alcohol consumption among active drinkers is increasing worldwide (1) and deaths related to ALD are expected to almost double from 2019 to 2040 [5].

The disease progression in NAFLD and ALD is associated with oxidative stress, mitochondrial dysfunction and lipid peroxidation [6]. These pathobiological changes may hold the key to the identification of biomarkers [5, 7, 8]. The current gold-standard diagnostic technique

is the liver biopsy (9,10). This procedure is time-consuming and expensive and carries a risk of complications [10]. Additional research is necessary to identify valid non-invasive biomarkers.

Mass-spectrometry (MS)-based proteomics is a promising method for the identification of accurate biomarkers. With MS-based analyses, it is possible to identify many proteins which may be involved in steatogenic, fibrinogenic or inflammatory pathways. These proteins can give a remarkable insight regarding pathophysiology and may be candidate biomarkers for diagnosis or disease activity of NASH or ASH. A strong proteomic analysis requires a thorough sample preparation, up-to-date instruments, and software capable of analyzing large amounts of data, see (Figure 2) [11]. The quality of these technologies is influenced by time and resources, which vary between research groups. This situation contributes to the lack of standardized methods of MS-based proteomic analyses, making results hard to reproduce. Several researchers are investigating possible biomarkers for diagnosis, monitoring or drug development of NAFLD and ALD. Due to the variation in methods and techniques, we aim to make a coherent literature review over current candidate protein biomarkers in NAFLD and ALD focusing on markers associated with steatosis, inflammation (steatohepatitis) and fibrosis and therefore with disease progression.

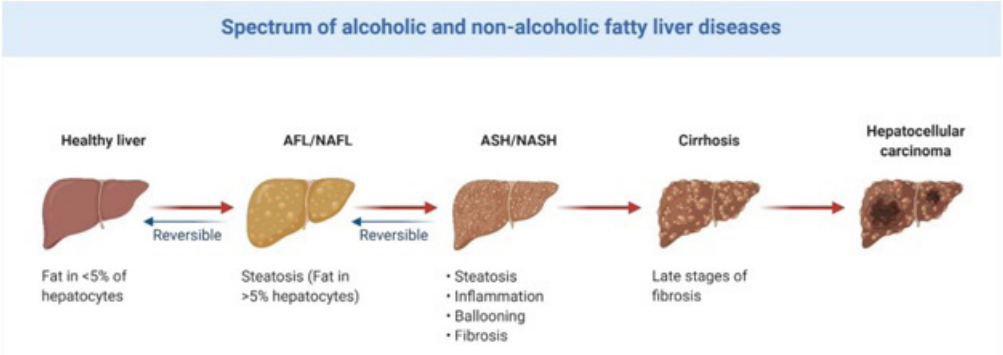

Figure 1: The natural spectrum of fatty liver diseases

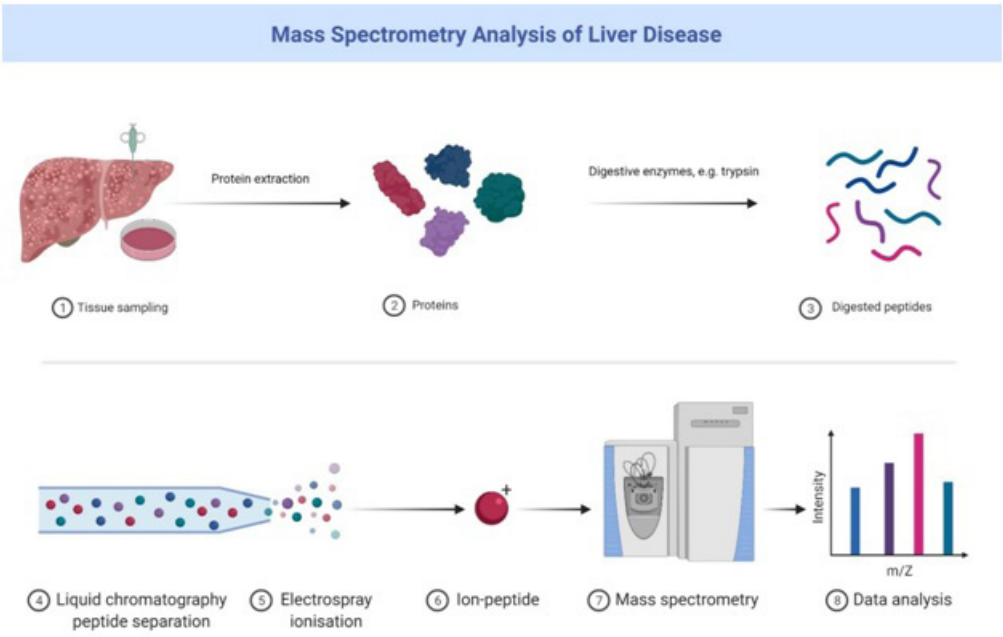

Figure 2: A simplified representation of the workflow of mass spectrometry analysis

3. Materials and Methods

A comprehensive search (conducted in January 2021) of the literature was performed in PubMed, Embase and Cochrane Library using the search phrase *"proteomics AND (NASH OR NASH cirrhosis"* and "p*roteomics AND (alcoholic steatohepatitis OR alcoholic liver fibrosis)*" for studies regarding MS-based proteomic analyses of histologically verified NAFLD and ALD describing the degree of inflammation and fibrosis in the liver of included patients or animals. Language was set to English. All publication types and study designs were considered.

4. Results

563 records were identified through database searching. After duplicates were removed, 366 articles remained, and 93 articles were assessed in full text. A total number of 8 articles were reviewed for this article.

4.1. Studies on NAFLD

One animal study and three human studies described MS-based analyses of NAFLD [12-15] see (Table 1). One study evaluated liver and plasma samples from Wistar rats [15], and two studies evaluated plasma samples in patients with histologically verified NAFLD [12, 14] or liver tissue from patients with NAFLD [13].

Hou et al. analyzed plasma samples 19 patients included in a study evaluating risk factors of NAFLD in patients with type 2 diabetes [12]. The analyses compared the proteomic profiles of ten patients with histologically verified NASH F0-F1 and nine patients with NAFLD F2-F4 [12].

Bell et al. included plasma samples from 69 patients with NAFLD and 16 controls with obesity (14). For patients, plasma was collected on the day of their scheduled liver biopsy. Based on histology, patients were classified into simple steatosis (n = 24), NASH F0-F2 (n= 23) and NASH F3-F4 (n=22). The control subjects were patients with obesity who had normal biochemistry, no evidence of insulin resistance or metabolic syndrome, and a normal liver ultrasound. Controls did not undergo a liver biopsy.

Rodríguez-Suárez et al. compared proteomic profiles in liver tissue obtained surgically from patients with NAFLD during bariatric surgery or patients without liver disease undergoing cholecystectomy [13]. The study included six patients with simple steatosis, six with NASH without fibrosis and six controls described as having normal liver biopsies.

Veyel et al. analysed plasma and liver tissue from Wistar rats (n=8) fed a NASH-inducing CDAA (choline-deficient L-amino-acid-defined) +1% cholesterol diet for twelve weeks. This was compared with plasma and liver tissue from control rats fed a (n=8) [15].

Table 1: Description of the human studies on NAFLD

Study	Population and intervention	Control	Pathology at timeframe	Candidate biomarkers	Tissue
Hou et al., 2020 (12)	N = 10 Biopsy-proven NASH F0-F1 N = 9 Biopsy-proven NASH F2-F4	None	NASH F0-F1 n = 10 NASH F2-F4 n= 9	Complement component C7	Blood
Rodriguez-Suarez et al., 2010 (13)	N = 6 Biopsy-proven steatosis N = 6 Biopsy-proven NASH grade 1	N = 6 Healthy controls, samples obtained during open cholecystectomy	Steatosis and NASH grade 1	Carbamoyl phosphate synthase 1 78 kDa glucose-regulated protein	Liver
Bell et al., 2010 (14)	N = 24 Biopsy-proven simple steatosis N = 23 Biopsy-proven NASH F0-F2 N = 24 Biopsy-proven simple steatosis N = 23 Biopsy-proven NASH F0-F2 N = 22 Biopsy-proven NASH F3-F4	N = 16 Obese controls, diagnosed by ultrasound and biochemical parameters	From simple steatosis to NASH F0-F4	Fibrinogen beta chain Paraoxonase 1 Prothrombin (fragment) Retinol binding protein 4 Transgelin 2 Complement component C7 Serum amyloid P component Lumican Insulin-like growth factor acid labile subunit CD5 antigen-like	Blood

Veyel et al., 2020 (15)	N = 8 Male Wistar rats CDAA+1% cholesterol diet	N = 8 CSAA diet	12 weeks Established fibrosis	Polymeric immunoglobulin receptor Complement component C7 Amyloid P component Serum Phospholipid transfer protein A disintegrin and metalloproteinase with thrombospondin motifs 2 Collagen type VI alpha 1 chain Collagen type VI alpha 2 chain Galectin-3-binding protein Clusterin Galectin-3-binding protein Clusterin Carboxypeptidase Q precursor	Blood and liver

Description of studies using mass spectrometry proteomics analyses on patients with NAFLD. CDAA: choline-deficient l-amino-acid, CSAA: choline-supplemented l-amino-acid.

4.2. Studies on ALD

One study analyzed the plasma proteomic profiles of patients with alcoholic cirrhosis [16] and four studies evaluated plasma and liver tissue from animals with ALD [17-20].

Maras et al. analyzed the plasma proteome of twenty patients with histologically verified severe alcoholic hepatitis and controls (number not reported) without evidence of liver disease (methods of assessment not reported) [16]. After performing MS-based proteomics on the subjects' blood, Paraoxonase-1 was left as the candidate biomarker and highlighted in the study. The animal studies used Lieber-DeCarli diets with varying lengths and concentrations of treatment [21]. For details on the animal studies, see (Table 2).

Bhopale et al. analyzed plasma and liver tissue from four animals with alcoholic steatohepatitis compared with four control animals [17]. Lee et al. investigated six mice where histology and immunohistochemistry revealed hepatic steatosis with activated stellate cells, myofibroblasts and macrophages [18]. The proteomic analyses were performed on liver tissue and compared with six control animals on a standard diet. Fernando et al. investigated six animals with hepatic steatosis on liver histology and expression of oxidative stress and inflammation on immunohistochemistry [19]. The proteomic analyses were performed on liver tissue and compared with six control animals. Banerjee et al. investigated the difference between four male and four female weight-matched animals [20]. After six weeks, liver histology and chloroacetate esterase staining showed hepatic fat accumulation with inflammation and necrosis, and significantly higher liver injury in females than males.

Table 2: Description of the animal studies on ALD

Study	Population and intervention	Control	Pathology at timeframe	Biomarker candidates	Tissue
Bhopale et al., 2017 (17)	N = 4 One-year old male hepatic ADH- deer mice Lieber-DeCarli diet initially 1g%, increased to 3.5g% over two weeks, than maintained at 3.5g% for additional 3 months	N = 4 Isocaloric liquid diet where ethanol calories were replaced with maltose-dextrin	3 months Histology and immunohistochemistry: Hepatic steatosis with infiltration of T-lymphocytes	Apolipoprotein E	Liver and blood

Lee et al., 2016 (18)	N = 6 Male C57BL/6J mice Lieber-DeCarli diet with 5g% W/v alcohol	N = 6 Standard diet	6 weeks Histology, immunohistochemistry and qRT-PCR analysis: Hepatic steatosis, increased expression of inflammatory genes, positive markers for activated stellate cells, myofibroblasts and macrophages.	Vitamin D-Binding Protein Ornithine aminotransferase, mitochondrial Phospatidylethanolamine-binding protein 1	Liver
Fernando et al., 2013 (19)	N = 6 Male Fischer 344 rats 6 weeks old Lieber-DeCarli liquid diet. Increased from 1% to 5% and then maintained at 5% for 3 months.	N = 6 Liquid diet with maltose-dextrin substituted for calories from ethanol	1 month and 3 months Histology and immunohistochemistry: 1 month: Hepatic steatosis 3 months: Progressive hepatic steatosis At both timeframes, expression of oxidative stress and inflammation.	Carbamoyl-phosphate synthase Alcohol dehydrogenase 1c Heat shock protein family a member 5 Betaine-homocysteine s-methyltransferase 2 D-dopachrome decarboxylase Peroxiredoxin 1 Cytochrome b5 type a Carbamoyl phosphate synthetase 1 Aldehyde dehydrogenase 2 Acyl-coa synthetase long chain family member 1	Liver
Banerjee et al., 2008 (20)	N = 4 female N = 4 male Weight matched Sprague-Dawley rats Lieber-DeCarli diet with 2% 2 days 4% 2 days and continued for 6 weeks + LPS	N = 4 female N = 4 male Isocaloric maltose-dextrin diet + LPS	6 weeks Histology and chloroacetate esterase staining: Male and female with hepatic fat accumulation, inflammation and necrosis, with higher liver injury in females than males	Peroxiredoxin 6 Heat Shock Protein 60 Ferritin heavy chain Ethylene responsive catalase Cytokeratin 19	Liver

Description of studies using mass spectrometry-based proteomics on animals with induced alcoholic liver disease. ADH-: Hepatic alcohol dehydrogenase depleted, LPS: lipopolysaccharide

4.3. Candidate Biomarkers

Two proteins (heat shock protein 60 and the keratin isoform family) were disregarded for further investigation, as they are likely to be products of the experimental setting or contamination [22]. Of the remaining candidate proteins, four were associated with inflammation or fibrosis in histologically verified NAFLD/ALD, see (Table 3 and 4).

Table 3: Candidate biomarkers in human studies

Protein	Studies	Population	Tissue	Up/Down-regulation	Expression and secretion
Carbamoyl phosphate synthetase 1 *Function*: Detoxification process, ammonia removal	Rodríguez-Suárez et al.	NASH human	Liver	Downregulated	Liver Not predicted to be secreted
Cytochrome b5 type a *Function*: Electron carrier	Rodríguez-Suárez et al.	NASH human	Liver	Downregulated	Liver Not predicted to be secreted

Paraoxonase-1 *Function*: Lipid metabolism	Bell et al Maras et al.	NASH human ASH human	Blood Blood	Downregulated Downregulated	Liver Secreted to blood
Lumican *Function*: Extracellular matrix protein	Bell et al.	NASH human	Blood	Upregulated	Placenta Not predicted to be secreted
CD5 antigen like *Function*: Regulator of lipid synthesis	Bell et al.	NASH human	Blood	N/A	Lymphoid tissue Secreted to blood
Alpha-1 acid glycoprotein precursor *Function*: Transport protein	Rodríguez-Suárez et al.	NASH human	Liver	Upregulated	Liver Secreted to blood

An overview over candidate biomarkers found in studies using mass spectrometry-based proteomics on patients with NAFLD.

Table 4: Candidate biomarkers in animal studies

Protein	Studies	Population	Tissue	Up/Down-regulation	Expression and secretion
PIGR *Function*: Immune response	Veyel et al.	NASH rat	Liver, blood	Upregulated	Gallbladder, intestine, salivary gland Not predicted to be secreted
Clusterin *Function*: Extracellular matrix protein	Veyel et al. Bhopale et al.	NASH rat ASH mice	Liver, blood Blood	Upregulated Downregulated	Liver, epididymis Secreted to blood
Peroxiredoxin isoforms *Function:* Detoxification process	Fernando et al. Banerjee et al.	ASH rat ASH rat	Liver Liver	Prx1 Down Prx6 Down	Low tissue specificity Not predicted to be secreted
Vit D binding protein *Function*: Transport protein	Lee et al. (2016)	ASH mice	Liver	Upregulated	Liver Secreted to blood
Ornithine aminotransferase *Function*: Amino acid synthesis	Lee et al. (2016)	ASH mice	Liver	Upregulated	Intestine Not predicted to be secreted
Phosphatidylethanolamine-binding protein 1 *Function*: Binding protein	Lee et al. (2016)	ASH mice	Liver	Upregulated	Liver Not predicted to be secreted
Cytochrome b5 type a *Function*: Electron carrier	Fernando et al. Bhopale et al.	ASH rat ASH mice	Liver Blood	Downregulated Upregulated	Liver Not predicted to be secreted

An overview over candidate biomarkers found in studies using mass spectrometry-based proteomics in animals with induced non-alcoholic steatohepatitis or alcoholic liver disease.

4.4. Proteins Related to Lipid Metabolism

The earliest research on PON1 investigated its properties as a hydrolyzer of insecticides from the organophosphorus (OP) family [23]. Plasma PON1 activity has been shown to vary in the general population due to major polymorphisms of the PON1-gene, which sparked researchers to investigate genetic susceptibility to OP poisoning [24]. Newer research shows similar patterns in PON1's association with coronary heart disease. A meta-analysis concluded that the R allele of this gene increases the risk of coronary heart disease [25], suggesting a possible genetic predisposition to coronary heart disease. PON1 is secreted from the liver and bound to HDL in serum in a compound together with clusterin and apolipoprotein A-I [26]. The enzyme is antiatherogenic due to its ability to prevent lipid peroxidation of LDL [25], and due to its association with HDL, which is protective of atherosclerosis. Low levels of PON1 independent of genotype have been associated with metabolic diseases, such as diabetes, hypercholesterolemia and non-alcoholic fatty liver disease [27, 28].

One study included patients with all stages of the NAFLD spectrum, from simple steatosis (n=24), NASH F0-F2 (n=23) to NASH F3-F4 (n=22). All patients had undergone a liver biopsy for diagnosis. Obese people without liver disease were included as control subjects (n=16) [14]. The main aim of the study was to produce biomarker panels for greater diagnostic accuracy. PON1 was decreased in all stages of NAFLD. Combined with another protein, prothrombin fragment, the diagnostic accuracy of differentiating healthy controls from patients in all stages of NAFLD was perfect (AUROC = 1.0). Another study reported PON1 in patients with biopsy-proven severe alcoholic hepatitis (n = 20) and in an unreported number of healthy controls [16]. Low plasma PON1 levels were inversely correlated with the severity of alcoholic hepatitis and termed a predictor of survival (28-day mortality). Other findings were increased levels of plasma oxidized LDL and increased macrophage lipid loading.

4.5. Proteins Related to Fibrosis Development

Clusterin/apolipoprotein J is expressed in nearly all tissues and body fluids, and has a wide range of functions depending on its location and molecular form [29]. When derived from the liver, clusterin has a protective anti-fibrotic and anti-steatotic effect, while adipocyte-derived clusterin expression is linked to increased insulin resistance and cardiovascular risk [30]. Clusterin was found in two animal models [15, 17] Veyel et al. found clusterin to be upregulated on a liver protein level and plasma protein level in mice with liver fibrosis caused by a NASH-inducing diet [15]. Bhopale et al. investigated mice with hepatic steatosis and inflammation due to administration of ethanol. Clusterin was found to be downregulated in blood [17].

Lumican is a proteoglycan which activates pro-fibrotic pathways and regulates assembly of collagen fibrils [31]. It is expressed in several tissues including the liver and is suggested to play a central role in hepatic fibrinogenesis [31, 32]. Lumican was found upregulated in blood samples from patients with NASH F3-F4 [14], and in blood samples from patients with alcoholic liver cirrhosis [33]. Lumican concentrations increase progressively with NAFLD disease stages, as observed in a study on obese patients with simple steatosis or NASH compared to obese controls [32]. Another study presented a correlation between lumican and hepatic collagen in NAFLD patients, and designated lumican a possible indirect marker for fibrosis development [34].

4.6. Proteins Related to Immune Response

Polymeric immunoglobulin receptor (PIGR), is a protein involved in mucosal immune response through mediation of IgA and IgM secretion [35]. This receptor-protein is found on the basolateral side of the mucosal epithelium and transports immunoglobulins, produced by plasma cells, through the epithelial cells to the apical end and releases them into the luminal space, after which it is cleaved [36]. The pathophysiological role of PIGR in liver pathology is unknown, and there is little research available on the subject. It has previously been linked to colorectal cancer, liver metastases, NAFLD and liver cirrhosis [35, 37, 38]. PIGR was found in one study on ten patients with alcoholic liver cirrhosis, showing increased levels in blood [33] Another study measured PIGR in both blood and liver from rats with NASH, where it was found increased in plasma but downregulated in the liver on an RNA level [15].

5. Discussion

This review aims to highlight the current research on biomarkers that may identify the progressive disease stages of NASH and ASH, in the hope of finding a common biomarker for inflammation and/or fibrosis. In the conducted literature search, four relevant proteins were found; PON1, clusterin, lumican and PIGR. PON1 was found decreased in all biopsy proven NAFLD patients of all stages compared with control subjects [14]. In addition, PON1 was also decreased in patients with biopsy proven alcoholic hepatitis compared with control subjects [16].

Twenty-five years ago, PON1 was found downregulated in patients with both type I and type II diabetes [39], and this finding has been replicated in patients with diabetes, NAFLD and metabolic syndrome [14, 16, 40-44]. The relationship between PON1 serum concentrations and NAFLD disease stages has not been clearly established. Several studies have shown that PON1 is downregulated in all stages of NAFLD and in alcoholic liver disease [14, 16, 42-44]. A paradox result was reported by van den Berg et al., who found that serum PON1 activity was maintained at normal levels in patients from a large cohort with "suspected NAFLD" [45]. The patients were diagnosed based on fatty liver index, a formula including several laboratory parameters. Hashemi et al. similarly found no significant difference in PON1 activity between NAFLD patients and healthy controls [46]. The NAFLD patients in this cohort were diagnosed based on laboratory results and sonography. Diagnostic methods and tools have differed over time, and these studies may be difficult to compare to the biopsy-verified patients in previously mentioned studies.

The main cause of death among patients with NAFLD is cardiovascular diseases [47]. PON1 has antiatherogenic effects, and the potential protective effect of PON1 in NAFLD should be further explored. Some studies have proposed using statins as prevention for conditions with increased lipid peroxidation, due to its antiatherogenic properties. Wysocka et al. found higher levels of PON1 activity in patients with diabetes and confirmed coronary heart disease when treated with a statin compared to the control group [48]. Samy et al. found an increase in serum PON1 activity in patients with NAFLD, when treated with atorvastatin [44]. Maras et al. conducted an in vitro study where THP-1-derived macrophages was treated with plasma samples from patients with severe alcoholic hepatitis (SAH) with or without substitution with recombinant PON1. The macrophages treated with SAH plasma increased the gene expression of markers of lipid uptake and lipid biosynthesis, and subsequent treatment with recombinant PON1 resulted in significantly reduced intracellular bodies and gene expression related to oxidative stress and inflammation (16). These findings suggest that higher levels of PON1 in conditions where it would naturally be depleted, may aid in prevention of diseases where lipid peroxidation is a driving force of disease development.

Clusterin was upregulated in mice with NASH and downregulated in mice with ethanol-induced steatosis and inflammation [15,17]. Clusterin can directly inhibit the activation of stellate cells through activation of the Smad3 pathway, which are central components of fibrosis development [49]. Seo et al. reported that up-regulation of the clusterin reduced pro-fibrotic pathways and macroscopic fibrosis in TAA-induced fibrotic mouse livers [49]. Similar results were found by Park et al., where overexpression of hepatic clusterin reduced steatosis, inflammation and fibrosis in MCD-diet induced NASH mice [50]. Although the form and function of clusterin is widely dependent on the tissue of which it derives, interesting treatment results was seen in mice with Alzheimer disease [51]. The cerebral beta-amyloidosis seen in Alzheimers disease was replicated in old mice, and when treated with intravenous recombinant ApoJ/Clusterin the levels of cerebral beta-amyloid was reduced [51].

Lumican concentrations increased progressively with liver damage in both NAFLD and ALD compared with control subjects [14, 33]. To determine the role of lumican in liver disease, Krishnan et al. assessed lumican expression in several presentations of liver disease [31]. Acute necroinflammation and chronic liver injury were induced in animals by injecting CCl4 intraperitoneally. Steatohepatitis with fibrosis was induced in animals through a fast-food diet. Transforming growth factor B1 (TGFB1) is a pro-fibrotic cytokine with increased expression in acute and chronic liver injury. To assess lumicans' possible role in pro-fibrotic pathways, Primary Human Hepatocytes were treated with TGFB1 in vitro. Lumican was upregulated in all of these presentations of liver disease [31].

In a study of 79 obese non-fibrotic NAFLD patients, lumican was found in higher concentrations in patients with pre-diabetes or metabolic syndrome than in patients with normal carbohydrate metabolism [52]. The same study reported that higher lumican levels presented a 3.9-fold increased risk of pre-diabetes [52]. This suggests that lumican and pro-fibrotic mechanisms may be activated in the very early stages of NAFLD, and lumican may be involved in increasing the fibrosis rate in patients with concomitant diabetes and NAFLD.

PIGR was increased in patients with alcoholic liver cirrhosis [33]. The role of PIGR in liver diseases is mostly unknown. Ai et al. established an association between PIGR expression and prognosis in patients with hepatocellular carcinoma (HCC) [53]. Newer research have confirmed the role of PIGR in HCC development through activation of pro-oncogenic pathways (54). PIGR also acts as a mediator of chronic viral hepatitis and indicator of liver metastasis development [53]. PIGR has recently been highlighted in a study by Niu et al., where it was significantly elevated in patients with NAFLD (170%) and in patients with cirrhosis (298%) compared to controls [35].

From an economical and societal perspective, finding a biomarker for ALD and NASH will have a great impact. Alcohol consumption among active drinkers is increasing globally (1), and deaths related to alcoholic liver disease are expected to almost double from 2019 to 2040, unless strong interventions are implemented (5). The number of NASH cases in the US is predicted to increase 63% from 2015 to 2030, rising to approximately 27 million cases in total (2). NAFLD is already projected to cost $100 billion annually in the US alone (7). Patients with cirrhosis have considerably reduced productivity and quality of life [55], however early intervention to prevent cirrhosis is cost-effective (2).

From a clinical perspective, diagnosis and surveillance with biomarkers are faster, cheaper and less resource-consuming than with a liver biopsy, and even with imaging methods. For the patient, risks and discomforts are markedly reduced and the number of days spent in hospital may be reduced.

Limitations of this review exist. As previously mentioned, methods of analysis vary between research groups, which makes comparison of results between the studies inconclusive. Three of the studies on human patients had a small sample size (from 6 to 19 participants), which impacts the statistical power of the study [12, 13, 33].

In conclusion, there is no current evidence supporting a single biomarker candidate, specifically related to the progressive stages of ALD and NAFLD. The proteins PON1, clusterin, lumican and PIGR may play various roles in the disease development and future research might enlighten the impact of these proteins in NASH and NAFLD. The relationship between NAFLD and diabetes, hypertension, dyslipidemia and heart disease seem important on a protein level, and future proteomic research might reveal the pathophysiology behind this concurrence.

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