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Review Article

Coagulation and Endothelial Dysfunction Associated with NAFLD: Current Status and Therapeutic Implications

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is closely related to insulin resistance, type 2 diabetes mellitus, and obesity. It is nowadays considered a multisystem disease with a strong association with cardiovascular disease and arterial hypertension, which interfere with changes in the coagulation system. Coagulation disorders are common in patients with hepatic impairment and are dependent on the degree of liver damage. Patients with NAFLD may have preserved overall hemostatic profile, but many studies suggest a trend toward a procoagulant state. Hypercoagulable state in NAFLD patients may even induce progression of hepatic injury. Endothelial dysfunction is present in the systemic and portal vein circulation in NAFLD patients, and platelets are being recognized as modulators of liver diseases through various mechanisms. Through a literature review, we discuss possible disorders in the coagulation cascade and fibrinolysis, endothelial dysfunction, and platelet abnormalities in patients with NAFLD. Considering the processes and mechanisms involved in the hemostatic abnormalities associated with NAFLD, directly related to liver disease or indirectly related through inflammatory processes and metabolic disorders, several potential therapeutic targets have been identified and reviewed here.

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Keywords: Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Insulin resistance; Coagulation; Endothelial dysfunction; Platelet dysfunction; Thrombosis.

Abbreviations: ADMA, asymmetric dimethyl-arginin; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; BMI, body mass index; CAD, coronary artery disease; CIMT, carotid artery intima-media thickness; CV, cardiovascular; CVD, cardiovascular disease; DOAC, direct oral anticoagulants; ED, endothelial dysfunction; eNOS, endothelial nitric oxide synthase; esRAGE, endogenous secretory ligand-receptor for advanced glycation-end-products; ETP, endogenous thrombin potential; F, factor; FCHL, familial combined hyperlipidemia; FMD, flow-mediated dilatation; GGT, gamma-glutamyl transferase; HCC, hepatocellular carcinoma; HOMA, homeostasis model assessment; HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high sensitivity C-reactive protein; ICAM, intercellular adhesion molecule; IL, interleukin; iNOS, inducible nitric oxide synthase; INR, international normalized ratio; IR, insulin resistance; LAL, lysosomal acid lipase; MPV, mean platelet volume; MS, metabolic syndrome; MTHFR, methylenetetrahydrofolate reductase; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NEFA, nonesterified fatty acids; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; OGTT, oral-glucose tolerance test; PAI-1, plasminogen activator inhibitor-1; PCOS, polycystic ovary syndrome; PDGF-β, platelet-derived growth factor beta; PDW, platelet distribution width; PT, prothrombin time; PTT, partial thromboplastin time; PTX3, pentraxin-related protein; RAGE, receptor for advanced glycation-end-product; ROS, reactive oxygen species; ROTEM, rotational thromboelastometry; SelP, selenoprotein P; SREBF, sterol regulatory element binding transcription factor; TAFI, thrombin activatable fibrinolysis inhibitor; TEG, thromboelastography; TFPI, tissue factor pathway inhibitor; TNF-α, tumor necrosis factor-α; t-PA, tissue plasminogen activator; T2DM, type 2 diabetes mellitus; uPA, urokinase plasminogen activator; US, ultrasound; VET, viscoelastic testing; VICAM, vascular cell adhesion molecule; vWF, von Willebrand factor; vWF:RCo, von Willebrand factor ristocetin cofactor activity; ω-3 PUFA, omega-3-polyunsaturated fatty acids.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease worldwide. It is the second most common reason for liver transplantation in the United States and is associated with significant mortality.[1] In parallel with global obesity and the metabolic syndrome epidemic, it is estimated that NAFLD affects approximately 25% of the adult population.[2,3]

NAFLD comprises a disease spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), which implies inflammation and hepatocyte ballooning degeneration with the development of progressive fibrosis and finally cirrhosis.[4,5] Of particular concern is a rising incidence of hepatocellular carcinoma (HCC) in NAFLD patients because of the high prevalence of the disease and the possibility of carcinoma development in the earlier stages of the disease, without significant fibrosis or cirrhosis, making screening virtually impossible.[6–9] The lack of HCC surveillance in non-cirrhotic NAFLD patients results in the advanced stage car-

cinoma detection with limited treatment options and poor outcomes.[10,11] However, despite significant liver-related morbidity, the most common cause of death in NAFLD patients is cardiovascular disease (CVD).[12–14]

NAFLD develops as a consequence of insulin resistance (IR), and is considered a hepatic manifestation of the metabolic syndrome, which recently led to a new concept of the metabolic dysfunction-associated fatty liver disease (MAFLD).[5,15] It is closely related to type 2 diabetes mellitus (T2DM) or impaired fasting glucose, dyslipidemia, obesity or increased waist circumference, and high blood pressure. Excessive calorie intake and progressive obesity lead to accumulation of body fat stores resulting in alterations in lipid metabolism and inflammation with consequent IR and changes in the post-receptor insulin metabolism pathways.[16,17]

Consequently, NAFLD is often considered a multisystem disease.[15] A strong correlation has been established with T2DM, CVD, arterial hypertension, and chronic kidney disease, but an increased risk has also been observed for NAFLD and various cancers, and neurological and chronic pulmonary diseases.[14,18–20] The link between NAFLD and the aforementioned disorders has not been fully elucidated, but special attention is given to low-grade chronic inflammation, which is the hallmark of NAFLD patients.

Changes in the gut microbiota with a persistent chronic inflammatory environment have been implicated in NAFLD pathogenesis and progression.[21,22] Lipopolysaccharides, bile acids, and short-chain fatty acids all induce liver inflammation, fibrosis and apoptosis by different mechanisms.[23] Furthermore, changes in the microbiota and aforementioned metabolites result in activation of toll-like receptors on endothelial cells, platelet activation, and platelet-neutrophil interaction with subsequent release of proinflammatory cytokines and prothrombotic factors that contribute to cell aggregation and thrombus formation.[24]

Various epidemiological, clinical, and animal studies have reported increased risk of atherosclerosis and arterial and venous thrombosis in NAFLD patients, and have suggested abnormalities in coagulation parameters, fibrinolytic process, endothelial and platelet dysfunction associated with IR. However, the use of diverse models and parameters for each of the investigated components of the hemostatic process makes comparisons almost impossible.[25]

Therefore, we searched published reports of alterations in coagulation parameters and endothelial or platelet dysfunction associated with NAFLD. We searched the PubMed database for English-language articles using the terms non-alcoholic fatty liver disease (or NAFLD) and coagulation, non-alcoholic fatty liver disease (or NAFLD) and endothelial dysfunction, non-alcoholic fatty liver disease (or NAFLD) and platelet dysfunction (or thrombocyte dysfunction). Another set of publications was identified by searching the literature cited in the retrieved articles. We excluded publications that were not focused on NAFLD and coagulation disorders, platelet, or endothelial dysfunction.

Large disease burden, significant and often devastating clinical consequences, and the possibility of prevention or therapeutic intervention in the underlying changes in the hemostatic process, make this problem clinically relevant and important.[25] This comprehensive review summarizes the current knowledge of alterations of several components of the hemostatic process associated with NAFLD, based on clinical trial results, the proposed mechanisms responsible for the changes, the clinical consequences, and potential therapeutic targets.

Coagulation changes associated with NAFLD

Hemostasis is a complex physiological process intended to control the bleeding resulting from a vascular injury. It can be divided into three phases. Primary hemostasis is responsible for the rapid formation of a platelet plug at the site of the damaged vessel wall. Contraction of the injured vessel and platelet activation and aggregation mediated by von Willebrand factor (vWF) occur in this initial phase. The second phase relies on activation of a coagulation cascade, in which complex interactions between several coagulation factors lead to their activation and result in the formation and deposition of fibrin fibers that stabilize the clot. The third phase is fibrinolysis, which refers to the activation of plasminogen and formation of plasmin, which is needed for the dissolution of the fibrin clot.[26]

Dysregulation of the coagulation cascade is common in chronic diseases of the liver, as it is responsible for the synthesis of most of the coagulation factors.[27] In patients with cirrhosis, impaired synthesis of the coagulation factors results in an imbalance of procoagulant and anticoagulant mechanisms, and the predominance of either can result in prolonged bleeding or thrombotic events. However, alterations in the coagulation system in most cirrhotic patients simultaneously affect both procoagulant and anticoagulant mechanisms such that the balance is restored and "rebalanced" hemostasis is achieved.[25,27] In the case of NAFLD situation is even more complex. Obesity, metabolic syndrome and NAFLD increase the risk of venous and arterial thrombosis.[28] A number of factors are responsible for the procoagulant state; chronic inflammation, IR, and imbalance of adipokines have been most commonly implicated.[29]

Many studies have investigated the impact of obesity, insulin resistance (IR) and NAFLD on the coagulation system. Some studies reported that, as in cirrhosis, the overall hemostatic profile is maintained in those with NAFLD, but most reported a trend toward a procoagulant state in NAFLD patients.[30] A stepwise progression of hemostatic abnormalities with the increasing severity of NAFLD has been described, with the smallest changes noted in patients with simple steatosis and more severe derangements following the development of NASH or cirrhosis.[31–33] Several studies suggested that prothrombotic features (increased activity of procoagulant factors VIII, IX, XI, and XII) in NAFLD patients correlated with liver fat content. Others found that changes in the plasma procoagulant profile (increased circulating VIII, IX, XI, vWF, and fibrinogen, hypofibrinolysis and a prothrombotic structure of fibrin clots) might be driven by obesity or IR, rather than by an increase in liver fat content.[30,34,35] The described alterations may reflect inflammation of adipose tissue and increased hepatic production of coagulation factors or their susceptibility to activation.[35]

The retrieved studies are highly variable regarding the population, methods used to establish the diagnosis of NAFLD, and the parameters used to assess the coagulation system, and are therefore difficult to compare. The study findings relevant to changes in the coagulation system of patients with NAFLD are summarized in Table 1.[30–48] The parameters and methods used to measure changes in the coagulation system are a significant challenge, not only in clinical studies, but also in everyday clinical practice. Routinely used coagulation tests such as prothrombin time, activated thromboplastin time, or measurement of individual pro- and anticoagulant factors, cannot assess the overall hemostatic profile of a patient because of the complex interactions that occur in the coagulation and fibrinolytic cascade in vivo. That is particularly important and evident in cirrhotic patients because disturbances of the coagulation cascade render common coagulation tests inadequate.[49] The use of thromboelastography (TEG), viscoelastic testing (VET), and rotational thromboelastometry (ROTEM) for comprehensive analysis of coagulation status is being extensively studied in clinical settings.[50–52] TEG may be usefull in NAFLD patients, because increased cardiovascular risk may be partially at-

Table 1. Changes in the coagulation system of patients with NAFLD

Methods	Study population	Findings and conclusions	Reference
Thrombin generation, plasma clot lysis, TAFI, prothrombin fragment 1+2, D-dimer, t-PA and PAI-1, activated TAFI (TAFIa/ai), and plasmin-α2-antiplasmin complex were measured	113 subjects with NAFLD diagnosed by ultrasound; participants in a study of chronic supplementation of nutraceutical mixture; results compared to healthy subjects	Thrombin generation parameters (ETP and peak thrombin activity) were significantly higher in NAFLD patients compared to the control group. Parameters suggest an increased procoagulant potential in NAFLD subjects	Cerletti C *et al*., 2020[37]
INR and serum concentrations of omentin, vaspin and irisin	25 patients with histologically diagnosed NAFLD	Negative correlation was found between INR and irisin concentration. Positive correlation was found between INR and vaspin concentration. Irisin had negative correlation with the grade of inflammation	Waluga M *et al*., 2019[38]
PT, APTT and activities of fibrinogen, vWF, FVII, FVIII, FIX, FXI, FXII and FXIII, D-dimer, vWF: RCo	92 subjects divided according to PNPLA3 genotype at rs738409 into those with (PNPLA3148MM/MI) and without (PNPLA3148II) the I148M variant, and based on median HOMA-IR into insulin-resistant 'IR' and insulin-sensitive 'IS' groups. NAFLD stage evaluated by biopsy or proton magnetic resonance spectroscopy (1 H-MRS)	Expression of proinflammatory genes in adipose tissue correlated positively with PT, circulating FVIII, FIX, FXI, vWR: RCo and fibrinogen. Expression of anti-inflammatory genes negatively correlated with PT (%), FIX and fibrinogen. Obesity/IR rather than an increase in liver fat is associated with a procoagulant plasma profile	Lallukka S *et al*., 2017[35]
Basal and agonist-induced platelet activation, plasma levels of markers of platelet activation and platelet adhesion regulators vWF and ADAMTS13, thrombomodulin-modified thrombin generation, thromboelastography, plasma fibrinolytic potential, clot permeability	68 patients with biopsy-proven NAFLD: simple steatosis (n=24), NASH (n=22) and NASH cirrhosis (n=22); 30 lean controls, 30 overweight controls (BMI >25 kg/m^2), and 15 patients with alcoholic cirrhosis	Hemostatic profile was comparable between patients with non-cirrhotic NAFLD and controls. Plasma fibrinolytic potential was decreased in overweight controls and non-cirrhotic NAFLD. Clot permeability was decreased in overweight controls and patients with NAFLD. Prothrombotic features (hypofibrinolysis and a prothrombotic structure of fibrin clot) in patients with NAFLD are likely driven by obesity	Potze W *et al*., 2016[30]
Pro- and anticoagulants plasma levels, thrombin generation assessed as endogenous thrombin potential (ETP) with and without thrombomodulin or Protac as protein C activators	113 patients with history of liver damage: steatosis (n=32), steatohepatitis (n=51), metabolic cirrhosis (n=30); 54 with alcoholic or viral cirrhosis and 179 controls	NAFLD was characterized by an increased FVIII, reduced protein C and procoagulant imbalance progressing from the less severe (steatosis) to the most severe form of the disease (metabolic cirrhosis)	Tripodi A *et al*., 2014[32]
Levels of PAI-1, vWF, uPA, thrombomodulin and TFPI	53 NAFLD patients with diagnosis established by ultrasonography in comparison with control group	Significant difference in vWF and TFPI levels between patient and control groups. No statistically significant difference was obtained in PAI-1, uPA and thrombomodulin levels. Level of vWF was positively correlated with the plasma TFPI levels in NAFLD patients	Bilgir O *et al*., 2014[39]
Fibrinogen concentrations and D-dimers measured before and after intervention	45 subjects with NAFLD diagnosed by ultrasound participated in a randomized study of three kinds of diets	Low calorie, low carbohydrate, soy containing diet decreased serum insulin level and serum fibrinogen	Kani AH *et al*., 2014[40]
Platelets, APTT, PT, FVII, FVIII, vWF, FXI, antithrombin III, protein C, activated protein C resistance, platelet function and PAI-1	273 obese patients with histologically proven NAFLD	Significant increase in PAI-1 levels were found through different stages of the NAFLD, with highest levels present in patients with severe steatohepatitis	Verrijken A *et al*., 2014[31]

(continued)

Table 1. *(continued)*

Methods	Study population	Findings and conclusions	Reference
Clot structure and fibrinolysis measurements	13 patients with PCOS and NAFLD diagnosed by ultrasonography and F-score for fibrosis; 10 patients with biopsy-confirmed NAFLD-compared to 12 patients with PCOS without NAFLD	No difference in blood clot structure and function between the patients with PCOS and NAFLD compared to PCOS without NAFLD	Dawson AJ *et al*., 2014[41]
PT and APTT, activities of vWF:RCo, FVII, FVIII, FIX, FXI, FXII, FXIII, fibrinogen and D-dimer concentrations	54 subjects with and 44 without NAFLD diagnosed by proton magnetic resonance spectroscopy	FVIII, FIX, FXI and FXII activities were increased in subjects with NAFLD and correlated with the liver fat. PT, vWF:RCo activity and fibrinogen were higher in subjects with NAFLD (difference disappeared after adjusting for age, gender and BMI)	Kotronen A *et al*., 2011[34]
Activity of protein C, antithrombin III, plasminogen, presence of lupus anticoagulant and anticardiolipin antibodies, protein C, activated protein S resistance, the presence of prothrombin G20210A polymorphism and factor V Leiden	60 patients with histologically diagnosed NAFLD compared with a historical control of 90 patients with chronic viral hepatitis B or C	Patients with NAFLD had higher mean levels of protein C, protein S, and plasminogen compared to chronic viral hepatitis, more frequently present anticardiolipin antibodies but less frequently protein S deficiency and the presence of thrombotic risk factors. Activated protein C resistance was present in 3% and prothrombin G20210A polymorphism in 7% of cases. Patients with NASH compared to fatty liver disease did not differ in the levels of any thrombophilic factor except for higher mean levels of IgG anticardiolipin antibodies	Papatheodoridis GV *et al*., 2009[42]
Clotting kinetics using thromboelastography (TEG)	28 patients with NAFLD (diagnosis based on histology in 18 subjects, or ultrasound in 10) and 22 healthy subjects	Patients with NAFLD showed increased clot strength and reduced clot lysis. Clot strength was positively associated with BMI in NAFLD. No association between clot kinetics and features of the metabolic syndrome or presence of type 2 diabetes was found	Hickman IJ *et al*., 2009[36]
Plasma PAI-1 concentrations and hepatic expression of PAI-1 mRNA were determined	12 histologically proven NAFLD patients and six controls	PAI-1 plasma concentrations and hepatic PAI-1 mRNA expression were significantly increased in NAFLD patients compared to controls	Thuy S *et al*., 2008[43]
Plasma concentration of fibrinogen and PAI-1	45 male patients with biopsy-proven NASH, 45 matched overweight men without steatosis on ultrasound and 45 healthy controls	Plasma concentrations of fibrinogen and PAI-1 activity were the highest in subjects with NASH, intermediate in overweight patients without steatosis, and the lowest in healthy controls	Targher G *et al*., 2008[44]
PAI-1 and TAFI	27 patients with biopsy-proven NASH and 18 healthy controls	Plasma PAI-1 levels were higher and mean plasma TAFI levels were lower in NASH patients compared to controls	Yener S *et al*., 2007[45]
PT or INR, partial thromboplastin time (PTT), fibrinogen, FVIII activity, deficiency in anti-thrombin, protein S, protein C, presence of lupus anticoagulant, activated protein C resistance with factor V Leiden mutation, G20210A mutation in the prothrombin gene and MTHFR mutation	15 patients with fatty liver; 15 with NASH; 14 with chronic viral hepatitis diagnosed by histology and liver technetium scan or ultrasound; 10 healthy controls	Activated protein C resistance and protein S were the most prevalent thrombotic risk factors in patients with NAFLD and chronic viral hepatitis. Prevalence of antithrombin III deficiency was more frequent in NAFLD as compared to chronic viral hepatitis. Patients with NASH had more decrease in protein S levels compared to the patients with fatty liver alone. Patients with severe fibrosis had markedly more decrease in protein S levels compared to patients without fibrosis. Protein C levels were higher in NASH patients regardless of fibrosis stage compared to patients with chronic viral hepatitis and correlated with the extent of fatty infiltration	Assy N *et al*., 2005[46]

(continued)

Table 1. *(continued)*

Methods	Study population	Findings and conclusions	Reference
Fibrinogen, vWF, PAI-1 activity	35 male patients with NAFLD (diagnosed by ultrasound or CT) and 65 controls	Plasma levels of fibrinogen, vWF and PAI-1 activity were significantly increased in subjects with NAFLD compared to control subjects without steatosis	Targher G *et al.*, 2005[47]
FVII clotting activity, PAI-1 activity and antigen, t-PA activity	31 male patients with NAFLD (diagnosis based on ultrasound) and 33 controls	FVII clotting activity, PAI-1 antigen and activity were significantly increased and t-PA activity significantly decreased in patients with liver steatosis as compared to those without steatosis. Changes were related to concomitant alterations in plasma triglyceride and insulin concentrations	Cigolini M *et al.*, 1996[48]

TAFI, thrombin activatable fibrinolysis inhibitor; t-PA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor-1; ETP, endogenous thrombin potential; PT, prothrombin time; APTT, activated partial thromboplastin time; vWF, von Willebrand factor; F, factor; vWF:RCo ,von Willebrand factor ristocetin cofactor activity; BMI , body mass index; NASH, non-alcoholic steatohepatitis; INR, international normalized ratio; TFPI, tissue factor pathway inhibitor; PCOS, polycystic ovary syndrome; HOMA-IR, homeostatic model assessment of insulin resistance; IR, insulin resistance; uPA, urokinase plasminogen activator; BMI, body mass index; TEG, thromboelastography; MTHFR , methylenetetrahydrofolate reductase.

tributed to altered clot kinetics resulting in lysis-resistant clots. Further studies are needed to substantiate those findings.[36] Although these assays are good for evaluating the global hemostatic profile, they are not widely accessible and are therefore underused in clinical practice.[50]

Endothelial dysfunction associated with NAFLD

The many roles of endothelial cells include providing normal blood flow through intact blood vessels, regulation of vascular tone (vasodilatation), modulation of prothrombotic and procoagulation processes by controlling the release and function of coagulation and fibrinolytic factors such as thrombomodulin, protein C and protein S, thrombin and antithrombin activation, tissue factor pathway inhibitor (TFPI), platelet activation and adhesion.[53] Nitric oxide (NO) is a substrate responsible for maintaining the proper function of endothelial cells. Physiological NO production is dependent on endothelial nitric oxide synthase (eNOS), which is largely specific to vascular endothelium. However, inducible nitric oxide synthase (iNOS) and neuronal NOS (nNOS) are other isoforms that can produce NO, but with different biological consequences.[54] Shear stress, vasoconstriction, and insulin promote NO production by eNOS and iNOS, but it has been shown that iNOS activation is more prominent in inflammation and can worsen IR and hyperglycemia, promote oxidative stress, and downregulate eNOS, contributing to endothelial dysfunction (ED).[55,56] eNOS activity is impaired in IR, which results in reduced NO production and subsequently in ED.[57] It has been shown that ED and IR have a reciprocal association connecting cardiovascular and metabolic diseases.[58] Moreover, insulin and inflammation influence vascular homeostasis via NO production, which maintains endothelial health by anti-inflammatory and antithrombotic activity.[59] In an extensive review of proinflammatory cytokines and adipokines in NAFLD, we reported that NAFLD should be regarded as a chronic proinflammatory state essential for the development of ED.[60]

Intrahepatic ED has been studied in animal models of NAFLD and other liver diseases.[61–65] Dysfunction of hepatic sinusoidal endothelial cells is essential for activation of hepatic stellate cells and Kupffer cells.[66,67] Sinusoidal microthrombus formation is promoted through secretion of several prothrombotic factors and receptors, as well as recruitment of neutrophils and platelets, which all contribute to parenchymal extinction and further fibrosis progression.[68,69] The severity of ED correlates with disease severity in the NAFLD spectrum, and indicates the severity of vascular wall damage.[70] Not only does NAFLD severity correlate with the progression of atherosclerosis, but the evidence also suggests that NAFLD is an independent risk factor for the occurrence and progression of CVD.[70,71] Thus, proper evaluation of ED and identification of the specific and sensitive markers of ED are essential for the prevention and reversal of both liver and systemic cardiovascular damage. Relevant clinical studies investigating ED associated with NAFLD are summarized in Table 2.[72–108]

Although many studies have shown a correlation between IR and NAFLD severity, no data on the involvement of intrahepatic vascular abnormalities in humans are available.[53,109] Although liver-specific markers of ED are lacking, it is prudent to investigate and recommend NAFLD treatment that modulates IR, as it might benefit sinusoidal endothelial cells, prevent further liver derangement, and improve perfusion within the steatotic liver .[110,111]

Platelet dysfunction associated with NAFLD

Recent evidence supports a paradigm shift recognizing

Table 2. ED and atherosclerosis in patients with NAFLD

Methods	Study population	Findings and conclusions	Reference
FcγRIIb levels	26 patients with biopsy-proven NAFLD	FcγRIIb expression levels correlated negatively with serum lipids, type 4 collagen and hyaluronic acid, which are involved in hepatic lipid metabolism disorder, fibrosis, and inflammation	Ishikawa T *et al*., 2019[73]
AGTR1 rs5186 A1166C variant, adipokine profile, inflammatory and ED markers, plasma lipoproteins, glucose, adipokines, MCP-1, calprotectin, and nuclear factor-κB activation in circulating mononuclear cells	78 biopsy-proven non-diabetic NAFLD patients and 314 controls	AGTR1 A1166C variant affects liver disease, IR, and ED in NAFLD, predicting a 9-year increase in CV disease risk and ED markers	Musso G *et al*., 2019[74]
Serum SelP levels, CIMT, FMD	93 patients with NAFLD (n=29 biopsy proven, n=64 proven by US staging) and 37 healthy controls	NAFLD patients had higher SelP, lower FMD and similar CIMT compared with controls. SelP, ESR and CRP were significantly higher and FMD lower in NASH compared with simple steatosis. FMD may be a better predictor for assessment of CVD risk when compared with cIMT	Cetindağlı I *et al*., 2017[75]
VCAM-1, placental growth factor, endoglin, vascular endothelial-cadherin	Obese male with biopsy-confirmed NAFLD patients undergoing bariatric surgery (n=61) and control patients (n=35)	Vascular endothelial-cadherin levels were higher and placental growth factor lower in NAFL and NASH patients compared to the controls. VCAM-1 was the only variable independently associated with significant fibrosis (>F2). VCAM-1 levels were able to accurately predict significant (>F2) fibrosis in NAFLD patients	Lafere S *et al*., 2017[76]
Platelet-derived phosphorylated-eNOS (p-eNOS), hepatic p-eNOS, FMD	54 biopsy-confirmed NAFLD patients (38,8% patients had NAFL and 61,7% NASH)	In NAFLD there was an impairment of eNOS and NAFL showed a higher impairment of eNOS phosphorylation in comparison to NASH ($p < 0.01$). The vascular response by FMD was worse in NASH as compared with NAFL. eNOS dysfunction observed in platelets and liver tissue did not match FMD	Persico M *et al*., 2017[77]
FMD, oxidative stress (Nox2 activation, serum isoprostanes and nitric oxide bioavailability (NOx))	19 biopsy-proven NASH patients, 19 NAFLD patients and 19 controls without signs of steatosis	NASH and FLD patients had higher Nox2 activity and isoprostanes levels and lower FMD and NOx, with a significant gradient between FLD and NASH compared to controls. Cocoa polyphenols improve endothelial function via Nox2 down-regulation in NASH patients	Loffredo L *et al*., 2017[78]
ED (strain-gauge plethysmography) after intra-arterial infusion of acetylcholine (ACh) and sodium nitroprusside	272 hypertensive patients with MS (consisting 93 NAFLD patients detected by calculating the noninvasive FLI)	MS and NAFLD hypertensive group showed a worse endothelium-dependent vasodilation compared with MS without NAFLD. NAFLD may be an early marker of ED in hypertensive's	Perticone M *et al*., 2016[79]
Brachial FMD	176 patients with US evaluated liver steatosis and 90 controls	Patients with grade 3 steatosis had significantly lower FMD values than those with grade 1 steatosis and controls. ED was associated with steatosis in patients with NAFLD	Sapmaz F *et al*., 2016[72]
PTX-3, ADMA, adiponectin, and hs-CRP	70 patients with biopsy-proven NAFLD and 70 healthy controls	Increased circulating PTX-3 was strongly associated with ED in subjects with NAFLD	Gurel H *et al*., 2016[80]
CIMT, FMD, pulse wave velocity measurement	61 male biopsy-proven NAFLD patients and 41 controls	NASH and NAFLD (with and without MS) patients had decreased FMD, increased CIMT and pulse wave velocity measurement than controls. NAFLD leads to increased risk of ED and atherosclerosis in adult male patients, independent of MS	Ozturk K *et al*., 2015[81]

(continued)

Table 2. *(continued)*

Methods	Study population	Findings and conclusions	Reference
ED (photoplethysmography), caspase-8	76 patients: 43 with MS (72.1% of them with NAFLD) and 33 controls	NAFLD patients had higher arterial stiffness, longer systolic duration, more pronounced ED. Higher caspase-8 levels may serve as a prognostic marker for the development of CVD and NAFLD	Drapkina OM *et al*., 2015[82]
FMD, peripheral arterial tonometry ratio, carotid-femoral pulse wave velocity	2,284 Framingham Heart Study participants without overt CVD (15,3% had NAFLD diagnosed with liver fat attenuation measured on computed tomography)	Greater liver fat was modestly associated with lower FMD, lower peripheral arterial tonometry ratio, higher carotid-femoral pulse wave velocity and higher mean arterial pressure	Long MT *et al*., 2015[83]
Brachial FMD	34 obese NAFLD patients (confirmed by MR imaging and spectroscopy) and 20 obese controls	NAFLD patients exhibited impaired FMD compared with controls, but could be improved by exercise training	Pugh CJ *et al*., 2014[84]
Fingertip pneumo-optic plethysmography in OSA patients	139 NAFLD (OSA) patients and 87 OSA patients (noninvasive blood tests were used to evaluate NAFLD- SteatoTest, NashTest, and FibroTest)	ED was more prominent in moderate or severe steatosis and borderline or possible NASH. The severity of nocturnal hypoxia was independently associated with steatosis. Preexisting obesity exacerbated the effects of nocturnal hypoxemia. NAFLD is a potential mechanism of ED in OSA	Minville C *et al*., 2014[85]
Endocan and high mobility group box 1 (ELISA), anti-endothelial cell antibodies (flow cytometry)	77 patients with US and/or fatty liver index diagnosed NAFLD	Severity of coronary artery disease in NAFLD positively correlated with endocan and negatively with high mobility group box 1 levels. Anti-endothelial cell antibodies were not significantly associated with coronary artery disease in NAFLD	Elsheikh E *et al*., 2014[86]
Serum VCAM-1, ICAM-1, MPO, adiponectin, PAI-1, SAP, SAA, E-selectin, and MMP-9	44 patients one year after liver transplantation compared to 22 biopsy-proven NASH patients and controls	Liver transplant patients and NASH patients had similar inflammatory and endothelial serum markers compared to the controls, lower IL-10 levels and higher IFNγ, E-selectin, serum VCAM-1 and ICAM-1 levels	Alvares-da-Silva MR *et al*., 2014[87]
Serum ICAM-1, endothelin-1 (ET-1), CIMT, brachial-ankle pulse wave velocity and ankle-brachial index	51 patients with biopsy-proven NAFLD	Serum levels of hs-CRP, sICAM-1, ET-1, CIMT and brachial-ankle pulse wave velocity were significantly higher in the NASH group than the NAFL group	Cao Y *et al*., 2014[88]
ADMA, brachial FMD	100 patients with NAFLD (US and fatty liver index, biopsy when available)	There was no significant difference in the serum ADMA concentration or FMD between the NAFLD and control groups	Sayki Arslan M *et al*., 2014[89]
Fetuin-A, ADMA, adiponectin, carotid atherosclerosis (cIMT)	115 patients with NAFLD (biopsy and US proven) and 74 healty controls	NAFLD group had higher fetuin-A, ADMA and cIMT, and lower adiponectin than control. Circulating fetuin-A in NAFLD was independently associated with ED and subclinical atherosclerosis	Dogru T *et al*., 2013[90]
Brachial and carotid artery FMD and CIMT	50 biopsy-proven NASH patients and 30 healthy controls	In patients with NASH, serum concentrations of GGT and ALT might have a predictive value for FMD and CIMT	Arinc H *et al*., 2013[91]

(continued)

Table 2. *(continued)*

Methods	Study population	Findings and conclusions	Reference
Endothelial progenitor cells	20 NAFLD patients (according to the degree of steatosis measured by US) and 20 individuals without NAFLD selected from the control group (n=96)	NAFLD group had increased levels of endothelial progenitor cells. Endothelial progenitor cells were associated with the severity of NAFLD	Gutiérrez-Grobe Y *et al*., 2013[92]
FMD, CIMT, lipids, insulin, C-peptide, and fasting blood glucose, HOMA-IR	161 patient, 117 US defined NAFLD patients	NAFLD is associated with impaired CIMT and FMD, which are early markers of atherosclerosis	Kucukazman M *et al*., 2013[93]
SREBF-2 polymorphism, endothelial adhesion molecules, plasma lipoproteins, adipokines, and cytokeratin-18 fragments	175 non-obese, non-diabetic participants without NAFLD or MS and NAFLD patients with liver biopsy	SREBF-2 polymorphism predisposes individuals to NAFLD and associated cardio-metabolic abnormalities. It also affects liver histology and glucose and lipid metabolism in NAFLD	Musso G *et al*., 2013[94]
Plasma ADMA, FMD, CIMT	51 biopsy-confirmed NAFLD patients and 21 controls	CIMT increase and FMD decrease was independent from MS and more evident in simple steatosis and NASH compared to controls. ADMA levels showed no significant difference in NAFLD and controls. NAFLD was associated with ED and increased earlier in patients with atherosclerosis compared to control subjects	Colak Y *et al*., 2013[95]
Plasma triglyceride-rich lipoproteins, oxidized low-density lipoproteins, adipokines, and cytokeratin-18 fragments	40 non-obese, non-diabetic, normolipidemic biopsy-proven NAFLD patients and 40 healthy subjects	Adipose IR, endothelial adhesion molecules, and hepatic IR progressively increased across NAFLD stages as well as cardio-metabolic parameters	Musso G *et al*., 2012[96]
OGTT fasting and 2 h, insulin, lipid profile, C-reactive protein, sICAM-1, VCAM-1, CIMT, FMD	40 NAFLD patients (fatty liver assessed by US) and 40 controls	NAFLD patients had a significantly greater degree of impairment in FMD, CIMT and higher levels of hs-CRP and sICAM-1. NAFLD was significantly associated with subclinical atherosclerosis and ED independent of obesity and MS	Thakur ML *et al*., 2012[97]
Plasma ADMA, glucose, lipids and insulin, HOMA-IR, CIMT	67 non-diabetic and normotensive biopsy-confirmed NAFLD patients and 35 healthy controls	Plasma ADMA levels were increased in subjects with NAFLD, independent from IR, liver histology and CV risk factors. Circulating ADMA may be an earlier marker of vascular damage with respect to CIMT in subjects with NAFLD	Dogru T *et al*., 2012[98]
Bone marrow-derived-endothelial progenitor cells	34 patients with US assessed NAFLD and 68 controls with suspected coronary artery disease	NAFLD patients had significantly decreased circulating endothelial progenitor cell levels, attenuated endothelial progenitor cell functions, and enhanced systemic inflammation compared to controls	Chiang CH *et al*., 2012[99]
CIMT, brachial FMD	84 patients with US defined NAFLD and 65 controls	Brachial FMD was significantly reduced in patients with NAFLD	Mohammadi A *et al*., 2011[100]
HOMA-IR, inflammatory adipokine score (IL-6, serum amyloid A, ICAM, adiponectin, and leptin), ED score (E-selectin, vascular cell adhesion molecule, vWF), and plasma levels of NEFA	434 subjects from the Cohort on Diabetes and Atherosclerosis Maastricht study. NAFLD diagnosed by increased ALT levels	IR, ED and NEFA, but not the MS or inflammatory adipokines, were significantly associated with plasma ALT. IR constitutes a key pathophysiological link between the MS and NAFLD, which may operate through adipose tissue-associated inflammation and ED and to a lesser extent through nonesterified fatty acids	Jacobs M *et al*., 2011[101]

(continued)

Table 2. *(continued)*

Methods	Study population	Findings and conclusions	Reference
Brachial FMD, hs-CRP, hs-IL6 and cell adhesion molecules, hepatocellular lipids, visceral and subcutaneous fat (MR spectroscopy)	28 obese children with NAFLD (MR spectroscopy elevated hepatic lipid content) compared with obese children with normal liver fat content	Increased hepatocellular lipid content was positively correlated to higher serum levels of hs-CRP and hs-IL6. No difference was found in vCAM and iCAM or FMD between groups	Weghuber D *et al*., 2011[102]
Endothelium-dependent vasodilation	40 hypertensive patients with laboratory and US proven NAFLD	Endothelium-dependent vasodilation was significantly reduced in hypertensive patients with NAFLD in comparison with hypertensive patients without NAFLD	Sciacqua A *et al*., 2011[103]
CIMT, carotid-femoral pulse wave velocity and FMD	23 biopsy-confirmed NAFLD patients and 28 controls subjects	NAFLD subjects had significantly higher carotid-femoral pulse wave velocity, CIMT and reduced FMD. NAFLD was associated with arterial stiffness and ED	Vlachopoulos C *et al*., 2010[104]
HOMA-IR >2, oxidative stress, soluble adhesion molecules (ICAM-1, VCAM-1 and E-selectin), and circulating adipokines (TNF-α, leptin, adiponectin, and resistin)	197 non-obese non-diabetic subjects (population-based cohort), NAFLD was assessed with US and elevated ALT (≥30 units/l in men and ≥20 units/l in women), 66% had liver biopsy	NAFLD independently predicted HOMA-IR, nitrotyrosine, and soluble adhesion molecules on logistic regression analysis. NAFLD was tightly associated with IR and markers of oxidative stress and ED and may help identify individuals with increased cardio-metabolic risk	Musso G *et al*., 2008[105]
Brachial endothelial-dependent dilatation (reactive hyperaemia), and endothelial-independent dilatation (sublingual nitrate)	15 NASH and 17 simple steatosis biopsy-confirmed NAFLD patients and 16 healthy subjects	Patients with NASH had worse ED compared with patients with simple steatosis and healthy subjects	Senturk O *et al*., 2008[106]
Immunoperoxidase stains for alpha-smooth muscle actin and CD31	Liver biopsies from 62 NAFLD patients, 21 HBV, and 19 HCV patients	CD31 was a marker of endothelial damage and sinusoidal capillary transformation particularly in NAFLD	Akyol G *et al*., 2005[107]
Flow-mediated vasodilation (FMV)	52 NAFLD cases (ALT≥1,5 ULN and US) and 28 age- and sex-matched controls	FMV was lower in NAFLD vs. controls and more pronounced in steatohepatitis than in simple fatty liver. The defect had to reside at the endothelium level because no differences were observed in flow-independent vasodilation	Villanova N *et al*., 2005[108]

ADMA, asymmetric dimethyl-arginin; ALT, alanine aminotransferase; CV, cardiovascular; CIMT, carotid artery intima-media thickness; ED, endothelial dysfunction; eNOS, endothelial nitric oxide synthase**;** FMD, flow-mediated dilatation; GGT- gamma-glutamyl transferase; HOMA, homeostasis model assessment; ICAM, intercellular adhesion molecule; IR, insulin resistance; IL, interleukin; MS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NAFL, non-alcoholic fatty liver; NASH – non-alcoholic steatohepatitis; NEFA, nonesterified fatty acids; NO, nitric oxide; OGTT, oral-glucose tolerance test; PTX3, pentraxin-related protein; SelP, selenoprotein P; SREBF, sterol regulatory element binding transcription factor; T2DM, type 2 diabetes mellitus; US, ultrasound; VICAM, vascular cell adhesion molecule; vW, von Wilebrand.

platelets as modulators of liver diseases including NAFLD via nonthrombotic mechanisms mediated by the interaction of receptors expressed on platelet surface with hepatic cells.[112] The mechanisms are not known, and are a matter of speculation. Reduced platelet count is undoubtedly linked with NAFLD, but the association seems to be restricted to more advanced fibrosis stages.[30,113] Mean platelet volume (MPV), an indicator of platelet activity and a potential indicator of prothrombotic state, is increased in patients with NAFLD, and may be associated with severity of inflammation and fibrosis.[114–116] However, interaction of the MPV with IR, the main culprit in the development and progression of NAFLD, remains an open question.[117,118] A study enrolling 100 biopsy-proven NAFLD patients found increased MPV in patients with increased steatosis, inflammation, ballooning, and fibrosis.[30] In patients with familial combined hyperlipidemia (FCHL) or metabolic syndrome, NAFLD was correlated with decreased activity of endogenous secretory ligand-receptor for advanced glycation-end-products (es-RAGE), pronounced atherothrombotic abnormalities, with increased CD40 ligand and endogenous thrombin potential (ETP) or decreased interleukin (IL) 10 and adiponectin, and an unfavorable genotypic cluster.[119] The pathogenesis of NAFLD thus represents a complex relationship of genetics, adipokine/cytokine secretion, oxidative stress and platelet activation.

The pathophysiology of NAFLD may involve lysosomal acid lipase (LAL), an enzyme that hydrolyzes triglycerides and cholesteryl esters in several types of hepatic cells and bone marrow-derived monocyte-macrophages. A decrease of platelet LAL has been associated with NAFLD severity in humans.[120] It increased lipophagy and cholesterol accumulation, promoting activation of platelet metabolism, migration, and aggregation, thus inducing their proinflammatory ability.[121,122]

A detailed study of the involvement of platelets in the pathophysiology, development, and progression of NAFLD in animal and cell-culture models reported that platelet number, activation, and aggregation were not affected by steatosis or IR, but were increased in NASH.[121] Platelet-derived glycoprotein Ib, primarily involved in platelet adhesion/activation but not platelet aggregation, was also involved in the pathogenesis of fibrosis and hepatocellular carcinoma.[121] The findings have been corroborated in recent human studies that described a protective role of aspirin in NAFLD progression.[123,124] The findings of the most relevant clinical studies of platelet dysfunction in NAFLD are summarized in Table 3.[77,114-118,120,123–128]

Discussion

NAFLD is a hepatic manifestation of the metabolic syndrome, and many NAFLD risk factors overlap with those predisposing patients to atherosclerosis and CVD. Studies investigating the natural history of the disease have shown that overall mortality is increased in NAFLD and is most often caused by CVD.[129–131] Increased mortality is probably multifactorial, depending on genetic predisposition, low-grade chronic inflammation, imbalance in proinflammatory cytokines and adipokines, oxidative stress, platelet abnormalities, ED and alteration of hemostasis, coagulation and fibrinolysis cascade, which have all been implicated in the development of CVD in NAFLD patients (Fig. 1). ED is the best characterized contributor to CVD in NAFLD, and leads to abnormal blood flow, vessel plaque formation and atherosclerosis.[131] ED is present in both the systemic and portal vein circulation in NAFLD patients.[72] Long-term follow-up demonstrated that NAFLD patients had a 55% increased risk of CVD after 30 years, and many studies have shown that NAFLD is an independent risk factor for CVD occurrence.[131,132,133]

Prothrombotic and procoagulant imbalance in patients with NAFLD results in an increased risk of clinically significant thrombotic events in the systemic circulation and portal venous system. Increased risk of portal vein thrombosis, venous thrombosis, and pulmonary embolism has been reported in liver transplant recipients with NASH.[134–137] A recent meta-analysis found a significant positive association between NAFLD and portal vein thrombosis.[138] Increased central obesity and leptin/adiponectin ratio in non-cirrhotic NAFLD patients were independent risk factors associated with development of portal vein thrombosis.[139]

A hypercoagulable state may induce progression of hepatic injury in NAFLD patients. Hepatocyte injury might be caused by microthrombosis of hepatic veins and arteries, abnormal circulation, liver congestion, ischemia and hepatocyte apoptosis, which consequently lead to NASH and even to cirrhosis.[25,140] Activation of the coagulation cascade was shown to be related to the development of steatohepatitis and fibrosis in an animal model of NAFLD.[141,142] Increased intrahepatic coagulation, as demonstrated by increased thrombin generation and hepatic fibrinogen deposition, was responsible for hepatic inflammation and fibrosis independent of lipid accumulation and injury, and was mediated by the thrombin receptor, protease activated receptor-1.[143] Obliterative lesions of small intrahepatic portal and hepatic veins that formed as a consequence of microthrombi formation were associated with parenchymal remodeling during fibrogenesis. Microthrombi formation was related to several thrombotic risk factors, including deficiency of protein C, antithrombin III and plasminogen, factor V Leiden mutation, and was associated with the extent of fibrosis in NAFLD and chronic viral hepatitis.[144]

Cardiovascular diseases and NAFLD share common underlying processes associated with metabolic syndrome and IR, which makes it difficult to distinguish the changes in coagulation associated with liver disease from those associated with IR, obesity, and metabolic risk factors. The function of liver parenchyma is usually well preserved in simple steatosis and NASH, but is impaired in advanced liver disease, resulting in possibly decreased procoagulant and anticoagulant factors levels and rebalanced hemostasis. The risk of thrombosis or hemorrhage in the cirrhotic liver greatly depends on individual patient factors.[25,136]

The available data on the changes in the coagulation system associated with NAFLD are conflicting. The main drawback is that most studies used noninvasive methods to establish an NAFLD diagnosis, and the grade of steatosis and fibrosis stage were rarely confirmed by liver biopsy. On the other hand, studies with biopsy-confirmed NAFLD and staging usually recruited few patients. Another problem is that most studies assayed the concentrations or activity of one or more components of the coagulation cascade that do not necessarily reflect their effects in vivo, and few assessed blood coagulation capacity, clot structure, or fibrinolysis. The differences are largely a consequence of the lack of widely available and reliable tests of procoagulant or fibrinolytic activity and the balance between procoagulant and anticoagulant drivers. However, use of thromboelastography could improve the care of these patients and assist in decision-making when introducing anticoagulant therapy or in the assessment of bleeding risk.[51,52]

Considering all the processes and mechanisms involved in the hemostatic abnormalities associated with NAFLD, directly related to liver disease or indirectly related through inflammatory processes and metabolic disorders, several potential therapeutic targets can be identified. Omega-3-polyunsaturated fatty acids (ω-3 PUFAs) are recognized as safe and effective modulators of systemic inflammation, and may be beneficial in preventing NAFLD/NASH progression.[145,146] A meta-analysis found that ω-3 PUFA improved

Table 3. Human studies of platelet dysfunction in patients with NAFLD

Methods	Study population	Findings and conclusions	Reference
Level of blood total lysosomal acid lipase (LAL) activity - intracellular platelet and leukocyte LAL were measured	Patients with NAFLD (n = 118), alcoholic (n = 116), and hepatitis C virus-related disease (n = 49), 103 controls with normal liver and 58 liver transplant recipients. A cross-sectional study	LAL in blood and platelets was reduced in NAFLD patients according to disease severity. High blood total LAL activity was associated with protection from NAFLD occurrence in subjects with metabolic and genetic predisposition. Low LAL in platelets and blood could play a pathogenetic role in NAFLD	Ferri F *et al*., 2020[120]
Association of aspirin use with prevalent NASH and fibrosis was investigated	Prospective cohort study of 361 adults with biopsy-confirmed NAFLD	Daily aspirin use was associated with less severe histologic features of NAFLD and NASH, and lower risk for progression to advanced fibrosis	Simon TG *et al*, 2019[123]
Relationship between PDGF-β serum concentration, platelets, liver fibrosis, and use of antiplatelet agents was investigated	505 patients included, 337 (67%) received antiplatelet agents and 134 (27%) had liver fibrosis	A protective association between the use of antiplatelet agents and occurrence of liver fibrosis was established	Schwarzkopf K *et al*., 2018[124]
Platelet number and function (MPV PDW, PT, PTT), lipid profile, hepatic aminotransferases, ferritin, and fasting blood sugar were evaluated	Case-control study with two groups of patients: 65 cases with NAFLD and 65 cases without NAFLD. NAFLD was diagnosed by ultrasound	Higher MPV was found to be significantly associated with NAFLD. No significant association was established regarding platelet count or PDW. MPV may be useful in follow-up of patients with NAFLD reglarding CV risk	Saremi Z *et al*., 2017[125]
eNOS function in platelets and liver specimens was determined	54 patients with liver biopsy-proven NAFLD	NAFLD patients exhibited eNOS dysfunction, which may contribute to a higher CV risk	Persico M *et al*, 2017[77]
Assessment of complete lipid profile, transaminases, HOMA-index, esRAGE, soluble CD40L, tumor necrosis factor-α, interleukin (IL)-6 and IL-10, adiponectin, leptin, and hs-CRP, polymorphisms related to inflammation and oxidative stress was performed	Observational study of 60 patients with vs. 50 without NAFLD diagnosed by ultrasound. Each group included patients with FCHL alone, metabolic syndrome (MS) alone, and FCHL plus MS	Among FCHL or MS patients, lower esRAGE and higher degree of atherothrombotic abnormalities coincided with the diagnosis of NAFLD. Interactions between genotype, adipokine secretion, oxidative stress and platelet/coagulative activation, could accelerate NAFLD occurrence	Santilli F *et al*., 2015[126]
MPV values in patients with and without NAFLD was determined	Meta-analysis of eight observational studies including 1,428 subjects (NAFLD=842 and non-NAFLD=586)	MPV was significantly higher in patients with NAFLD, indicating the presence of increased platelet activity in those patients	Madan SA *et al*., 2016[115]
Relation of MPV values with NAFLD and IR was investigated	54 patients with histologically proven NAFLD and 41 healthy age-matched control subjects	MPV was increased in NAFLD patients. MPV was not correlated with the increase of IR in NAFLD patients. MPV was not related with inflammation and steatosis degree, hepatocellular ballooning and fibrosis in NAFLD patients	Celikbilek M *et al*., 2013[118]
Assessment of MPV values	100 consecutive patients undergoing liver biopsy for clinical suspicion of NAFLD. Patients were divided into three groups: NASH (n=52), simple steatosis (n=25), and normal histology (n=21)	Higher MPV was associated with histologic severity of liver injury and inflammation in patients with biopsy-proven NAFLD	Alkhouri N *et al*., 2012[116]
Assessment of MPV values	6,499 healthy subjects (age range 20–65 years) recruited in Seoul. A cross-sectional study	After adjustment for confounding variables, the prevalence of NASH was significantly higher according to increased MPV values. There was a significant association between NASH and MPV in obese study population	Shin WY *et al*., 2011[114]

(continued)

Table 3. *(continued)*

Methods	Study population	Findings and conclusions	Reference
Assessment of MPV values	Retrospective study of 128 obese adolescents divided in two groups: patients with NAFLD and patients without NAFLD + control group	MPV was significantly higher in obese adolescents, NAFLD patients. MPV was significantly higher in patients with IR. There was a positive correlation between MPV and HOMA-IR. MPV was inversely correlated with HDL cholesterol and platelet count	Arslan N *et al.*, 2010[117]
Assessment of MPV values and CIMT	60 biopsy-proven NAFLD subjects and 54 healthy controls	No significant correlation was found between MPV and CIMT. No difference in MPV values between subjects with NAFLD and controls was established	Kilciler G *et al.*, 2010[127]
Assessment of MPV values	70 patients with NAFLD and 60 healthy controls. NAFLD diagnosis established by ultrasound and blood testing	Patients with NAFLD had higher MPV, lower platelet count and higher body mass index	Ozhan H *et al.*, 2010[128]

CIMT, carotid artery intima-media thickness; CV, cardiovascular; eNOS, endothelial nitric oxide synthase; HOMA-IR, homeostatic model assessment for insulin resistance; hs-CRP, high sensitivity C-reactive protein; IL, interleukin, FCHL, familial combined hyperlipidemia; IR, insulin resistance; LAL, lysosomal acid lipase; MPV, mean platelet volume, PDW, platelet distribution width; MS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NASH- non-alcoholic steatohepatitis; PDGF-β, platelet-derived growth factor beta; PT, prothrombin time, PTT, partial thromboplastin time; RAGE, advanced glycation-end-product.

plasma lipids, triglycerides, and markers of liver injury but without significant improvement of liver histology.[145] ω-3 and ω-6 PUFAs are essential components of platelet phospholipid membranes, and ω-3 and ω-6 PUFA supplementation has been used for a long time for prevention of CVD.[147] The benefits of ω-3 PUFAs on platelet function may prevent thrombotic incidents, tissue ischemia, liver tissue remodulation, and disease progression.[25,148]

Statins reduce cholesterol biosynthesis by inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase. They also have anti-inflammatory, antioxidant and antifibrotic activity, all of which may benefit NASH treatment.[149,150] Among their pleiotropic effects, antiatherogenic properties are of special interest regarding prevention of atherothrombotic events in NASH patients.[150,151] Statins have antioxidative, antiproliferative and anti-inflammatory properties that can improve endothelial function and reduce the risk of sudden cardiac death and deep vein thrombosis.[152] There is also evidence that statins may improve other components of NAFLD, including steatosis and liver fibrosis, but they are currently not indicated for the treatment of NAFLD.[5,150] However, statins are often prescribed to patients with NAFLD and metabolic syndrome as lipid-lowering agents and for prevention of cardiovascular events.[150] In patients with CAD and increased liver enzymes, statins have been associated with reduced cardiovascular mortality and improvement in liver function tests.[153] Statins have positive effects on ED, which is also present in hepatic sinusoids. The parenchymal extinction hypothesis suggests that microthrombosis of hepatic sinusoids triggers inflammation and liver fibrosis, and is possibly mediated by derangements in the coagulation cascade, including protein C deficiency, increased factor VIII expression and thrombin activation.[154] Statins increase protein C activity and decrease formation of von Willebrand factor and generation of thrombin by coagulation cascade activation, thus preventing activation of hepatic stellate cells and fibrosis progression.[155,156] In animal models, statins protect against thrombosis of the portal vein and hepatic sinusoids as well as against ischemic hepatitis. The mechanisms include anti-inflammatory activity and decreased release of von Willebrand factor, which is a key prothrombotic factor in the cirrhotic liver.[150]

Fenofibrate is a peroxisome proliferator-activated receptor-α agonist with beneficial effects in reducing triglycerides and decreasing synthesis of apolipoproteins. In addition, fenofibrate has shown anti-inflammatory properties that have been associated with reduced hepatic expression of tumor necrosis factor-α (TNF-α), decreased macrophage infiltration following inhibition of liver expression of monocyte chemoattractant protein-1, intercellular adhesion molecule-1 and vascular adhesion molecule-1.[157] Peroxisome proliferator-activated receptor-α gene expression differently affects lipid metabolism and inflammation in apolipoprotein E2 knock-in mice.[158] Peroxisome proliferator-activated receptor-α agonists also have beneficial effects on tissue microcirculation.[157] By inhibiting various mediators of vascular damage, inflammation, lipotoxicity, and reactive oxygen species formation, they prevent ED, thrombosis, and consequent microvascular complications. In the liver, their antioxidant activity can help reduce necrosis, inflammation, and fibrosis, but the data are inconclusive.[157]

Microbiota composition and metabolites such as lipopolysaccharides, bile acids, and short-chain fatty acids are important regulators of liver and systemic metabolism.[159] Dysbiosis of gut microbiota may be another link between NASH and coagulation disorders. Various strategies have attempted to modify gut microbiota and NASH development including antibiotic treatment, fecal transplantation, and alteration of gut metabolites. New and emerging treatments aimed at influencing both NASH development and coagu-

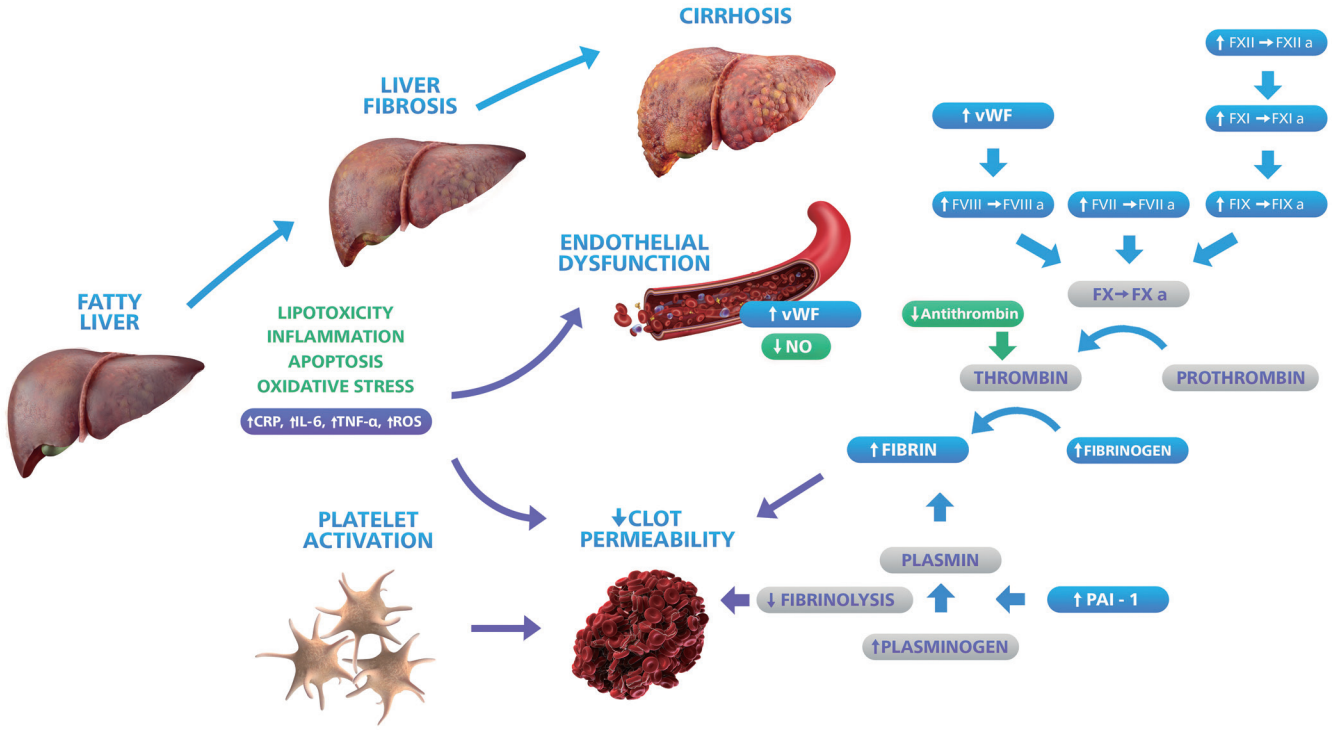

Fig. 1. Possible alterations in the coagulation cascade, fibrinolysis, platelet and ED associated with non-alcoholic fatty liver disease, with mechanisms responsible for potential prothrombotic and procoagulant imbalance in patients with NAFLD. CRP, C-reactive protein; F, factor; IL-6, interleukin 6; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; vWF, von Willebrand factor.

lation disorders include sevelamer. It is a hydrophilic bile acid sequestrant that was found to alter liver fibrosis by improving the intestinal barrier and fecal excretion of lipopolysaccharides and reducing the concentration of lipopolysaccharides in the liver.[23,160] Positive effects of sevelamer on endothelial and vascular function, as well as on reduction of atherosclerotic risk have been confirmed in many studies in chronic kidney disease patients. Human studies on sevelamer in NASH patients and its presumed positive effect on associated coagulation disorders are eagerly awaited.[161]

The available data on the alterations in the hemostatic mechanisms associated with metabolic syndrome and NAFLD suggest the evaluation of possible anticoagulation and antiplatelet therapy in these patients. Patients with advanced cirrhosis treated by enoxaparin had lower incidence of portal vein thrombosis, lower rates of hepatic decompensation, and improved survival.[162] Although the study included patients with various etiologies of cirrhosis, the possibility of preventing portal vein thrombosis and slowing the rate of disease progression by thromboprophylaxis seems intriguing. On the other hand, administration of anticoagulation therapy is complicated by derangements in the coagulation cascade associated with advanced liver disease. Updated clinical practice recommendations of the American Society of Gastroenterology (AGA), consider direct-acting anticoagulants (DOACs), relatively safe and effective in stable cirrhosis. In patients with advanced stages of liver disease more evidence is needed. Warfarin is allowed in highly selected patients with Child-Turcotte-Pugh class C and thrombotic incidents, although anticoagulation in general is not recommended in such patients.[49]

The benefit of antiplatelet agents in preventing liver fibrosis was investigated in a prospective cohort study of patients at high risk of liver fibrosis and cardiovascular events.[124] The study showed a protective effect of antiplatelet agents (acetyl salicylic acid and P2Y12 receptor antagonists including clopidogrel, prasugrel, or ticagrelor) on liver fibrosis assessed by transient elastography. Furthermore, implementation of antiplatelet therapy (aspirin/clopidogrel, ticagrelor) reduced fibrosis and NASH-induced HCC possibly by reducing intrahepatic platelet infiltration and platelet–immune cell interaction, mitigating cytokine driven liver damage.[112] There is a lack of evidence, with gaps in our knowledge to allow recommendations on that topic, but it seems prudent to conclude that anti-inflammatory, antiaggregation and anticoagulation therapy could prove beneficial, given the overlap of metabolic syndrome and liver disease, with derangements in several components of hemostasis, and CVD.

Conclusions

There is evidence that endothelial vascular dysfunction, platelet abnormalities, and alterations in factors involved in the coagulation cascade and fibrinolysis may all contribute to a prothrombotic state in patients with NAFLD. The alterations could be triggered by low-grade chronic inflammation. Involvement of multiple organs and organ systems results in high morbidity and mortality of NAFLD patients.

We have just started to scratch the surface of numerous possibilities revealed by the role of platelets, ED, and changes in the coagulation system and liver damage in terms of treatment, and management of NAFLD. As some data suggest that hypercoagulability is associated with NAFLD, it would be prudent to estimate benefits from anticoagulant and/or antiaggregation therapy in patients with NAFLD.

Currently available data of the role of platelets and alterations in the coagulation system are scarce and often conflicting, underlining the emerging need for larger, pro-

spective studies with well-defined patient groups and comprehensive tests for the assessment of hemostatic profiles. Identification of cytokines and/or receptors involved in platelets, immune cell reactions, mediators of ED, and triggers of coagulation abnormalities responsible for liver damage and adverse cardiovascular outcomes could be extremely useful for developing new pharmacological and therapeutic options. Future studies recruiting more patients with a well-defined NAFLD diagnosis and stage, careful selection of methods and clinically valuable indicators of individual hemostatic profiles are eagerly awaited.

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Conflict of interest

The authors have no conflict of interest related to this publication.

Author contributions

LVJ contributed to the conception and design of the study, collecting data, drafting and revising the manuscript critically. DO, AM, IBC, MCB and SSS were involved in collecting data, drafting, and writing the manuscript. All authors read and approved the final manuscript.

References

[1] Gadiparthi C, Spatz M, Greenberg S, Iqbal U, Kanna S, Satapathy SK, *et al*. NAFLD epidemiology, emerging pharmacotherapy, liver transplantation implications and the trends in the United States. J Clin Transl Hepatol 2020;8(2):215–221. doi:10.14218/JCTH.2020.00014, PMID:32832402.
[2] Duvnjak M, Lerotić I, Baršić N, Tomašić V, Virović Jukić L, Velagić V. Pathogenesis and management issues for nonalcoholic fatty liver disease. World J Gastroenterol 2007;13(34):4539–4550. doi:10.3748/wjg.v13.i34.4539, PMID:17729403.
[3] Geisler CE, Renquist BJ. Hepatic lipid accumulation: cause and consequence of dysregulated glucoregulatory hormones. J Endocrinol 2017;234:R1–R21. doi:10.1530/JOE-16-0513, PMID:28428362.
[4] Geier A, Tiniakos D, Denk H, Drauner M. From the origin of NASH to the future of metabolic fatty liver disease. Gut 2021;70:1570–1579. doi:10.1136/gutjnl-2020-323202.
[5] European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64(6):1388–1402. doi:10.1016/j.jhep.2015.11.004, PMID:27062661.
[6] Baffy G, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. J Hepatol 2012;56(6):1384–1391. doi:10.1016/j.jhep.2011.10.027, PMID:22326465.
[7] Huang DQ, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2021;18(4):223–238. doi:10.1038/s41575-020-00381-6, PMID:33349658.
[8] Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, *et al*. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. JAMA Oncol 2017;3(4):524–548. doi:10.1001/jamaoncol.2016.5688, PMID:27918777.
[9] European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol 2018;69(1):182–236. doi:10.1016/j.jhep.2018.03.019, PMID:29628281.
[10] Mittal S, El-Serag HB, Sada YH, Kanwal F, Duan Z, Temple S, *et al*. Hepatocellular carcinoma in the absence of cirrhosis in United States veterans is associated with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2016;14:124–131.e1. doi:10.1016/j.cgh.2015.07.019, PMID:26196445.
[11] Sanyal A, Poklepovic A, Moyneur E, Barghout V. Population-based risk factors and resource utilization for HCC: US perspective. Curr Med Res Opin 2010;26:2183–2191. doi:10.1185/03007995.2010.506375, PMID:20666689.
[12] Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413–1419. doi:10.1016/s0016-5085(99)70506-8, PMID:10348825.
[13] Danford CJ, Lai M. NAFLD: a multisystem disease that requires a multidisciplinary approach. Frontline Gastroenterol 2019;10(4):328–329. doi:10.1136/flgastro-2019-101235, PMID:31682642.
[14] Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol 2015;62:S47–64. doi:10.1016/j.jhep.2014.12.012, PMID:25920090.
[15] Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, *et al*. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol 2020;73(1):202–209. doi:10.1016/j.jhep.2020.03.039, PMID:32278004.
[16] Hazlehurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver disease and diabetes. J Metabol 2016;65(8):1096–1108. doi:10.1016/j.metabol.2016.01.001, PMID:26856993.
[17] Bhatt HB, Smith RJ. Fatty liver disease in diabetes mellitus. Hepato Biliary Surg Nutr 2015;4(2):101–108. doi:10.3978/j.issn.2304-3881.2015.01.03, PMID:26005676.
[18] Sanna C, Rosso C, Marietti M, Bugianesi E. Non-alcoholic fatty liver disease and extra-hepatic cancers. Int J Mol Sci 2016;17(5):717. doi:10.3390/ijms17050717, PMID:27187365.
[19] Lombardi R, Fargion S, Fracanzani AL. Brain involvement in non-alcoholic fatty liver disease (NAFLD): A systematic review. Dig Liver Dis 2019;51(9):1214–1222. doi:10.1016/j.dld.2019.05.015, PMID:31176631.
[20] Botello-Manilla AE, López-Sánchez GN, Chávez-Tapia NC, Uribe M, Nuño-Lámbarri N. Hepatic steatosis and respiratory diseases: a new panorama. Ann Hepatol 2021;24:100320. doi:10.1016/j.aohep.2021.100320, PMID:33549735.
[21] Wieland A, Frank DN, Harnke B, Bambha K. Systematic review: microbial dysbiosis and nonalcoholic fatty liver disease. Aliment Pharmacol Ther 2015;42(9):1051–1063. doi:10.1111/apt.13376, PMID:26304302.
[22] Loomba R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, *et al*. Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. Cell Metab 2017;25(5):1054–1062.e5. doi:10.1016/j.cmet.2017.04.001, PMID:28467925.
[23] Stojsavljevic-Shapeski S, Duvnjak M, Virovic-Jukic L, Hrabar D, Smircic Duvnjak L. New drugs on the block - emerging treatments for nonalcoholic steatohepatitis. J Clin Transl Hepatol 2021;9(1):51–59. doi:10.14218/JCTH.2020.00057, PMID:33604255.
[24] Hasan RA, Koh AY, Zia A. The gut microbiome and thromboembolism. Thromb Res 2020;189:77–87. doi:10.1016/j.thromres.2020.03.003, PMID:32192995.
[25] Virović-Jukić L, Stojsavljević-Shapeski S, Forgač J, Kukla M, Mikolašević I. Non-alcoholic fatty liver disease - a procoagulant condition? Croat Med J 2021;62(1):25–33. doi:10.3325/cmj.2021.62.25, PMID:33660958.
[26] Gale AJ. Continuing education course #2: current understanding of hemostasis. Toxicol Pathol 2011;39(1):273–280. doi:10.1177/0192623310389474, PMID:21119054.
[27] Northup PG, Caldwell SH. Coagulation in liver disease: a guide for the clinician. Clin Gastroenterol Hepato 2013;11(9):1064–1074. doi:10.1016/j.cgh.2013.02.026, PMID:23506859.
[28] Spinosa M, Stine JG. Nonalcoholic fatty liver disease-evidence for a thrombophilic state? Curr Pharm Des 2020;26(10):1036–1044. doi:10.2174/1381612826662001311015 53, PMID:32003679.
[29] Nakamura K, Fuster JJ, Walsh K. Adipokines: A link between obesity and cardiovascular disease. J Cardiol 2014;63(4):250–259. doi:10.1016/j.jjcc.2013.11.006, PMID:24355547.
[30] Potze W, Siddiqui MS, Boyett SL, Adelmeijer J, Daita K, Sanyal AJ, *et al*. Preserved hemostatic status in patients with non-alcoholic fatty liver disease. J Hepatol 2016;65(5):980–987. doi:10.1016/j.jhep.2016.06.001, PMID:27302378.
[31] Verrijken A, Francque S, Mertens I, Prawitt J, Caron S, Hubens G, *et al*. Prothrombotic factors in histologically proven nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology 2014;59(1):121–129. doi:10.1002/hep.26510, PMID:24375485.
[32] Tripodi A, Fracanzani AL, Primignani M, Chantarangkul V, Clerici M, Mannucci PM, *et al*. Procoagulant imbalance in patients with non-alcoholic fatty liver disease. J Hepatol 2014;61(1):148–154. doi:10.1016/j.jhep.2014.03.013, PMID:24657400.
[33] Targher G, Bertolini L, Scala L, Zenari L, Lippi G, Franchini M, *et al*. Plasma PAI-1 levels are increased in patients with nonalcoholic steatohepatitis. Diabetes Care 2007;30(5):e31–32. doi:10.2337/dc07-0109, PMID:17468361.
[34] Kotronen A, Joutsi-Korhonen L, Sevastianova K, Bergholm R, Hakkarainen A, Pietiläinen KH, *et al*. Increased coagulation factor VIII, IX, XI and XII activities in non-alcoholic fatty liver disease. Liver Int 2011;31(2):176–183. doi:10.1111/j.1478-3231.2010.02375.x, PMID:21134109.
[35] Lallukka S, Luukkonen PK, Zhou Y, Isokuortti E, Leivonen M, Juuti A, *et al*. Obesity/insulin resistance rather than liver fat increases coagulation factor activities and expression in humans. Thromb Haemost 2017;117(2):286–294. doi:10.1160/TH16-09-0716, PMID:27929200.
[36] Hickman IJ, Sullivan CM, Flight S, Campbell C, Crawford DH, Masci PP, *et al*. Altered clot kinetics in patients with non-alcoholic fatty liver disease. Ann Hepatol 2009;8(4):331–338. PMID:20009132.
[37] Cerletti C, Colucci M, Storto M, Semeraro F, Ammollo CT, Incampo F, *et al*. Randomised trial of chronic supplementation with a nutraceutical mixture in subjects with non-alcoholic fatty liver disease. Br J Nutr 2020;123(2):190–197. doi:10.1017/S0007114519002484, PMID:31554528.
[38] Waluga M, Kukla M, Kotulski R, Zorniak M, Boryczka G, Kajor M, *et al*. Omentin, vaspin and irisin in chronic liver diseases. J Physiol Pharmacol 2019;70(2):277–285. doi:10.26402/jpp.2019.2.11, PMID:31356179.
[39] Bilgir O, Bilgir F, Bozkaya G, Calan M. Changes in the levels of endothelium-derived coagulation parameters in nonalcoholic fatty liver dis-

ease. Blood Coagul Fibrinolysis 2014;25(2):151–155. doi:10.1097/MBC.0000000000000009, PMID:24317388.
[40] Kani AH, Alavian SM, Esmaillzadeh A, Adibi P, Azadbakht L. Effects of a novel therapeutic diet on liver enzymes and coagulating factors in patients with non-alcoholic fatty liver disease: A parallel randomized trial. Nutrition 2014;30(7-8):814–821. doi:10.1016/j.nut.2013.11.008, PMID:24984998.
[41] Dawson AJ, Sathyapalan T, Smithson JA, Vince RV, Coady AM, Ajjan R, *et al*. A comparison of cardiovascular risk indices in patients with polycystic ovary syndrome with and without coexisting nonalcoholic fatty liver disease. Clin Endocrinol (Oxf) 2014;80(6):843–849. doi:10.1111/cen.12258, PMID:23746214.
[42] Papatheodoridis GV, Chrysanthos N, Cholongitas E, Pavlou E, Apergis G, Tiniakos DG, *et al*. Thrombotic risk factors and liver histologic lesions in non-alcoholic fatty liver disease. J Hepatol 2009;51(5):931–938. doi:10.1016/j.jhep.2009.06.023, PMID:19726097.
[43] Thuy S, Ladurner R, Volynets V, Wagner S, Strahl S, Königsrainer A, *et al*. Nonalcoholic fatty liver disease in humans is associated with increased plasma endotoxin and plasminogen activator inhibitor 1 concentrations and with fructose intake. J Nutr 2008;138(8):1452–1455. doi:10.1093/jn/138.8.1452, PMID:18641190.
[44] Targher G, Bertolini L, Rodella S, Lippi G, Franchini M, Zoppini G, *et al*. NASH predicts plasma inflammatory biomarkers independently of visceral fat in men. Obesity (Silver Spring) 2008;16(6):1394–1399. doi:10.1038/oby.2008.64, PMID:18369343.
[45] Yener S, Akarsu M, Demir T, Akinci B, Sagol O, Bayraktar F, *et al*. Plasminogen activator inhibitor-1 and thrombin activatable fibrinolysis inhibitor levels in non-alcoholic steatohepatitis. J Endocrinol Invest 2007;30(10):810–819. doi:10.1007/BF03349221, PMID:180775282.
[46] Assy N, Bekirov I, Mejritsky Y, Solomon L, Szvalb S, Hussein O. Association between thrombotic risk factors and extent of fibrosis in patients with non-alcoholic fatty liver diseases. World J Gastroenterol 2005;11(37):5834–5839. doi:10.3748/wjg.v11.i37.5834, PMID:16270394.
[47] Targher G, Bertolini L, Scala L, Zoppini G, Zenari L, Falezza G. Non-alcoholic hepatic steatosis and its relation to increased plasma biomarkers of inflammation and endothelial dysfunction in non-diabetic men. Role of visceral adipose tissue. Diabet Med 2005;22(10):1354–1358. doi:10.1111/j.1464-5491.2005.01646.x, PMID:16176196.
[48] Cigolini M, Targher G, Agostino G, Tonoli M, Muggeo M, De Sandre G. Liver steatosis and its relation to plasma haemostatic factors in apparently healthy men - role of the metabolic syndrome. Thromb Haemost 1996;76(1):69–73. PMID:8819254.
[49] O'Leary JG, Greenberg CS, Patton HM, Caldwell SH. AGA Clinical Practice Update: Coagulation in Cirrhosis. Gastroenterology 2019;157(1):34–43.e1. doi:10.1053/j.gastro.2019.03.070, PMID:30986390.
[50] Davis JPE, Northup PG, Caldwell SH, Intagliata NM. Viscoelastic testing in liver disease. Ann Hepatol 2018;17(2):205–213. doi:10.5604/01.3001.0010.8635, PMID:29469043.
[51] Olson JC. Thromboelastography-guided blood product use before invasive procedures in cirrhosis with severe coagulopathy: A randomized controlled trial. Clin Liver Dis 2019;13(4):102–105. doi:10.1002/cld.749, PMID:31061702.
[52] Premkumar M, Mehtani R, Divyaveer S, Kajal K, Kulkarni AV, Ahmed S, *et al*. Clinical validation of global coagulation tests to guide blood component transfusions in cirrhosis and ACLF. J Clin Transl Hepatol 2021;9(2):210–219. doi:10.14218/JCTH.2020.00121, PMID:34007803.
[53] Van Hinsbergh VWM. Endothelium - Role in regulation of coagulation and inflammation. Semin Immunopathol 2012;34(1):93–106. doi:10.1007/s00281-011-0285-5, PMID:21845431.
[54] Wilcox JN, Subramanian RR, Sundell CL, Tracey WR, Pollock JS, Harrison DG, *et al*. Expression of multiple isoforms of nitric oxide synthase in normal and atherosclerotic vessels. Arterioscler Thromb Vasc Biol 1997;17(11):2479–2488. doi:10.1161/01.atv.17.11.2479, PMID:9409218.
[55] Fujimoto M, Shimizu N, Kunii K, Martyn JA, Ueki K, Kaneki M. A role for iNOS in fasting hyperglycemia and impaired insulin signaling in the liver of obese diabetic mice. Diabetes 2005;54(5):1340–1348. doi:10.2337/diabetes.54.5.1340, PMID:15855318.
[56] Chauhan SD, Seggara G, Vo PA, Macallister RJ, Hobbs AJ, Ahluwalia A. Protection against lipopolysaccharide-induced endothelial dysfunction in resistance and conduit vasculature of iNOS knockout mice. FASEB J 2003;17(6):773–775. doi:10.1096/fj.02-0668fje, PMID:12586741.
[57] Potenza MA, Marasciulo FL, Chieppa DM, Brigiani GS, Formoso G, Quon MJ, *et al*. Insulin resistance in spontaneously hypertensive rats is associated with endothelial dysfunction characterized by imbalance between NO and ET-1 production. Am J Physiol Heart Circ Physiol 2005;289(2):H813–822. doi:10.1152/ajpheart.00092.2005, PMID:15792994.
[58] Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 2006;113(15):1888–1904. doi:10.1161/CIRCULATIONAHA.105.563213, PMID:16618833.
[59] Montagnani M, Ravichandran LV, Chen H, Esposito DL, Quon MJ. Insulin receptor substrate-1 and phosphoinositide-dependent kinase-1 are required for insulin-stimulated production of nitric oxide in endothelial cells. Mol Endocrinol 2002;16(8):1931–1942. doi:10.1210/me.2002-0074, PMID:12145346.
[60] Stojsavljević S, Gomerčić Palčić M, Virović Jukić L, Smirčić Duvnjak L, Duvnjak M. Adipokines and proinflammatory cytokines, the key mediators in the pathogenesis of nonalcoholic fatty liver disease. World J Gastroenterol 2014;20(48):18070–18091. doi:10.3748/wjg.v20.i48.18070, PMID:25561778.
[61] Pasarín M, La Mura V, Gracia-Sancho J, García-Calderó H, Rodríguez-Vilarrupla A, García-Pagán JC, *et al*. Sinusoidal endothelial dysfunction precedes inflammation and fibrosis in a model of NAFLD. PLoS One 2012;7(4):e32785. doi:10.1371/journal.pone.0032785, PMID:22509248.
[62] Ijaz S, Yang W, Winslet MC, Seifalian AM. The role of nitric oxide in the modulation of hepatic microcirculation and tissue oxygenation in an experimental model of hepatic steatosis. Microvasc Res 2005;70(3):129–136. doi:10.1016/j.mvr.2005.08.001, PMID:16203017.
[63] Pasarín M, Abraldes JG, Rodríguez-Vilarrupla A, La Mura V, García-Pagán JC, Bosch J. Insulin resistance and liver microcirculation in a rat model of early NAFLD. J Hepatol 2011;55(5):1095–1102. doi:10.1016/j.jhep.2011.01.053, PMID:21356259.
[64] Bosch J, Abraldes JG, Fernández M, García-Pagán JC. Hepatic endothelial dysfunction and abnormal angiogenesis: new targets in the treatment of portal hypertension. J Hepatol 2010;53(3):558–567. doi:10.1016/j.jhep.2010.03.021, PMID:20561700.
[65] Iwakiri Y. Endothelial dysfunction in the regulation of cirrhosis and portal hypertension. Liver Int 2012;32(2):199–213. doi:10.1111/j.1478-3231.2011.02579.x, PMID:21745318.
[66] Francque S, Laleman W, Verbeke L, Van Steenkiste C, Casteleyn C, Kwanten W, *et al*. Increased intrahepatic resistance in severe steatosis: endothelial dysfunction, vasoconstrictor overproduction and altered microvascular architecture. Lab Invest 2012;92(10):1428–1439. doi:10.1038/labinvest.2012.103, PMID:22890552.
[67] Miyao M, Kotani H, Ishida T, Kawai C, Manabe S, Abiru H, *et al*. Pivotal role of liver sinusoidal endothelial cells in NAFLD/NASH progression. Lab Invest 2015;95(10):1130–1144. doi:10.1038/labinvest.2015.95, PMID:26214582.
[68] Lafoz E, Ruart M, Anton A, Oncins A, Hernández-Gea V. The endothelium as a driver of liver fibrosis and regeneration. Cells 2020;9(4):929. doi:10.3390/cells9040929, PMID:32290100.
[69] Hilscher MB, Sehrawat T, Arab JP, Zeng Z, Gao J, Liu M, *et al*. Mechanical stretch increases expression of CXCL1 in liver sinusoidal endothelial cells to recruit neutrophils, generate sinusoidal microthombi, and promote portal hypertension. Gastroenterology 2019;157(1):193–209.e9. doi:10.1053/j.gastro.2019.03.013, PMID:30872106.
[70] Fargion S, Porzio M, Fracanzani AL. Nonalcoholic fatty liver disease and vascular disease: state of-the-art. World J Gastroenterol 2014;20(37):13306–13324. doi:10.3748/wjg.v20.i37.13306, PMID:25309067.
[71] Low Wang CC, Goalstone ML, Draznin B. Molecular mechanisms of insulin resistance that impact cardiovascular biology. Diabetes 2004;53(11):2735–2740. doi:10.2337/diabetes.53.11.2735, PMID:15504952.
[72] Sapmaz F, Uzman M, Basyigit S, Ozkan S, Yavuz B, Yeniova A, *et al*. Steatosis grade is the most important risk factor for development of endothelial dysfunction in NAFLD. Medicine (Baltimore) 2016;95(14):e3280. doi:10.1097/MD.0000000000003280, PMID:27057890.
[73] Ishikawa T, Yokoyama H, Matsuura T, Fujiwara Y. Fc gamma RIIb expression levels in human liver sinusoidal endothelial cells during progression of non-alcoholic fatty liver disease. PLoS One 2019;14(1):e0211543. doi:10.1371/journal.pone.0211543, PMID:30695042.
[74] Musso G, Saba F, Cassader M, Paschetta E, De Michieli F, Pinach S, *et al*. Angiotensin II type 1 receptor rs5186 gene variant predicts incident NAFLD and associated hypertension: Role of dietary fat-induced pro-inflammatory cell activation. Am J Gastroenterol 2019;114(4):607–619. doi:10.14309/ajg.0000000000000154, PMID:30920415.
[75] Cetindağlı I, Kara M, Tanoglu A, Ozalper V, Aribal S, Hancerli Y, *et al*. Evaluation of endothelial dysfunction in patients with nonalcoholic fatty liver disease: Association of selenoprotein P with carotid intima-media thickness and endothelium-dependent vasodilation. Clin Res Hepatol Gastroenterol 2017;41(5):516–524. doi:10.1016/j.clinre.2017.01.005, PMID:28760353.
[76] Lefere S, Van de Velde F, Devisscher L, Bekaert M, Raevens S, Verhelst X, *et al*. Serum vascular cell adhesion molecule-1 predicts significant liver fibrosis in non-alcoholic fatty liver disease. Int J Obes (Lond) 2017;41(8):1207–1213. doi:10.1038/ijo.2017.102, PMID:28461687.
[77] Persico M, Masarone M, Damato A, Ambrosio M, Federico A, Rosato V, *et al*. Non alcoholic fatty liver disease and eNOS dysfunction in humans. BMC Gastroenterol 2017;17(1):35. doi:10.1186/s12876-017-0592-y, PMID:28264657.
[78] Loffredo L, Baratta F, Ludovica P, Battaglia S, Carnevale R, Nocella C, *et al*. Effects of dark chocolate on endothelial function in patients with non-alcoholic steatohepatitis. Nutr Metab Cardiovasc Dis 2017;28(2):143–149. doi:10.1016/j.numecd.2017.10.027, PMID:29329924.
[79] Perticone M, Cimellaro A, Maio R, Caroleo B, Sciacqua A, Sesti G, *et al*. Additive effect of non-alcoholic fatty liver disease on metabolic syndrome-related endothelial dysfunction in hypertensive patients. Int J Mol Sci 2016;17(4):456. doi:10.3390/ijms17040456, PMID:27023537.
[80] Gurel H, Genc H, Celebi G, Sertoglu E, Cicek AF, Kayadibi H, *et al*. Plasma pentraxin-3 is associated with endothelial dysfunction in non-alcoholic fatty liver disease. Eur Rev Med Pharmacol Sci 2016;20(20):4305–4312. PMID:27831642.
[81] Ozturk K, Uygun A, Guler AK, Demirci H, Ozdemir C, Cakir M, *et al*. Nonalcoholic fatty liver disease is an independent risk factor for atherosclerosis in young adult men. Atherosclerosis 2015;240(2):380–386. doi:10.1016/j.atherosclerosis.2015.04.009, PMID:25875390.
[82] Drapkina OM, Deeva TA, Ivashkin VT. Evaluation of endothelial function and estimation of the degree of apoptosis in patients with metabolic syndrome and non-alcoholic fatty liver disease. Ter Arkh 2015;87(5):76–83. Russian-doi:10.17116/terarkh201587576-83, PMID:26155623.
[83] Long MT, Wang N, Larson MG, Mitchell GF, Palmisano J, Vasan RS, *et al*. Non-alcoholic fatty liver disease and vascular function: cross-sectional analysis in the Framingham heart study. Arterioscler Thromb Vasc Biol 2015;35(5):1284–1291. doi:10.1161/ATVBAHA.114.305200, PMID:25745056.
[84] Pugh CJ, Spring VS, Kemp GJ, Richardson P, Shojaee-Moradie F, Umpleby AM, *et al*. Exercise training reverses endothelial dysfunction in nonalcoholic

fatty liver disease. Am J Physiol Heart Circ Physiol 2014;307(9):H1298–1306. doi:10.1152/ajpheart.00306.2014, PMID:25193471.
[85] Minville C, Hilleret MN, Tamisier R, Aron-Wisnewsky J, Clement K, Trocme C, *et al*. Nonalcoholic fatty liver disease, nocturnal hypoxia, and endothelial function in patients with sleep apnea. Chest 2014;145(3):525–533. doi:10.1378/chest.13-0938, PMID:24264333.
[86] Elsheikh E, Younoszai Z, Otgonsuren M, Hunt S, Raybuck B, Younossi ZM. Markers of endothelial dysfunction in patients with non-alcoholic fatty liver disease and coronary artery disease. J Gastroenterol Hepatol 2014;29(7):1528–1534. doi:10.1111/jgh.12549, PMID:25587619.
[87] Alvares-da-Silva MR, de Oliveira CP, Stefano JT, Barbeiro HV, Barbeiro D, Soriano FG, *et al*. Pro-atherosclerotic markers and cardiovascular risk factors one year after liver transplantation. World J Gastroenterol 2014;20(26):8667–8673. doi:10.3748/wjg.v20.i26.8667, PMID:25024624.
[88] Cao Y, Li L. Relationship of non-alcoholic steatohepatitis with arterial endothelial function and atherosclerosis. Zhonghua Gan Zang Bing Za Zhi 2014;22(3):205–208. doi:10.3760/cma.j.issn.1007-3418.2014.03.012, PMID:24824123.
[89] Sayki Arslan M, Turhan S, Dincer I, Mizrak D, Corapcioglu D, Idilman R. A potential link between endothelial function, cardiovascular risk, and metabolic syndrome in patients with non-alcoholic fatty liver disease. Diabetol Metab Syndr 2014;6:109. doi:10.1186/1758-5996-6-109, PMID:25960770.
[90] Dogru T, Genc H, Tapan S, Aslan F, Ercin CN, Ors F, *et al*. Plasma fetuin-A is associated with endothelial dysfunction and subclinical atherosclerosis in subjects with nonalcoholic fatty liver disease. Clin Endocrinol 2013;78(5):712–717. doi:10.1111/j.1365-2265.2012.04460.x, PMID:22676641.
[91] Arinc H, Sarli B, Baktir AO, Saglam H, Demirci E, Dogan Y, *et al*. Serum gamma glutamyl transferase and alanine transaminase concentrations predict endothelial dysfunction in patients with non-alcoholic steatohepatitis. Ups J Med Sci 2013;118(4):228–234. doi:10.3109/03009734.2013.814734, PMID:23829707.
[92] Gutiérrez-Grobe Y, Gavilanes-Espinar JG, Masso-Rojas FA, Sánchez-Valle V, Páez-Arenas A, Ponciano-Rodríguez G, *et al*. Metabolic syndrome and nonalcoholic fatty liver disease. The role of endothelial progenitor cells. Ann Hepatol 2013;12(6):908–914. doi:10.3748/wjg.v22.i43.9488, PMID:24114821.
[93] Kucukazman M, Ata N, Yavuz B, Dal K, Sen O, Deveci OS, *et al*. Evaluation of early atherosclerosis markers in patients with nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol 2013;25(2):147–151. doi:10.1097/MEG.0b013e32835a58b1, PMID:23085576.
[94] Musso G, Bo S, Cassader M, De Michieli F, Gambino R. Impact of sterol regulatory element-binding factor-1c polymorphism on incidence of nonalcoholic fatty liver disease and on the severity of liver disease and of glucose and lipid dysmetabolism. Am J Clin Nutr 2013;98(4):895–906. doi:10.3945/ajcn.113.063792, PMID:23985808.
[95] Colak Y, Senates E, Yesil A, Yilmaz Y, Ozturk O, Doganay L, *et al*. Assessment of endothelial function in patients with nonalcoholic fatty liver disease. Endocrine 2013;43(1):100–107. doi:10.1007/s12020-012-9712-1, PMID:22661277.
[96] Musso G, Cassader M, De Michieli F, Rosina F, Orlandi F, Gambino R. Nonalcoholic steatohepatitis versus steatosis: adipose tissue insulin resistance and dysfunctional response to fat ingestion predict liver injury and altered glucose and lipoprotein metabolism. Hepatology 2012;56(3):933–942. doi:10.1002/hep.25739, PMID:22684858.
[97] Thakur ML, Sharma S, Kumar A, Bhatt SP, Luthra K, Guleria R, *et al*. Nonalcoholic fatty liver disease is associated with subclinical atherosclerosis independent of obesity and metabolic syndrome in Asian Indians. Atherosclerosis 2012;223(2):507–511. doi:10.1016/j.atherosclerosis.2012.06.005, PMID:22748277.
[98] Dogru T, Genc H, Tapan S, Ercin CN, Ors F, Aslan F, *et al*. Elevated asymmetric dimethylarginine in plasma: an early marker for endothelial dysfunction in non-alcoholic fatty liver disease? Diabetes Res Clin Pract 2012;96(1):47–52. doi:10.1016/j.diabres.2011.11.022, PMID:22189171.
[99] Chiang CH, Huang PH, Chung FP, Chen ZY, Leu HB, Huang CC, *et al*. Decreased circulating endothelial progenitor cell levels and function in patients with nonalcoholic fatty liver disease. PLoS One 2012;7(2):e31799. doi:10.1371/journal.pone.0031799, PMID:22359630.
[100] Mohammadi A, Sedani HH, Ghasemi-Rad M. Evaluation of carotid intima-media thickness and flow-mediated dilatation in middle-aged patients with nonalcoholic fatty liver disease. Vasc Health Risk Manag 2011;7:661–665. doi:10.2147/VHRM.S26011, PMID:22140316.
[101] Jacobs M, van Greevenbroek MM, van der Kallen CJ, Ferreira I, Feskens EJ, Jansen EH, *et al*. The association between the metabolic syndrome and alanine amino transferase is mediated by insulin resistance via related metabolic intermediates (the Cohort on Diabetes and Atherosclerosis Maastricht [CODAM] study). Metabolism 2011;60(7):969–975. doi:10.1016/j.metabol.2010.09.006, PMID:21040936.
[102] Weghuber D, Roden M, Franz C, Chmelik M, Torabia S, Nowotny P, *et al*. Vascular function in obese children with non-alcoholic fatty liver disease. Int J Pediatr Obes 2011;6(2):120–127. doi:10.3109/17477161003792580, PMID:20950125.
[103] Sciacqua A, Perticone M, Miceli S, Laino I, Tassone EJ, Grembiale RD, *et al*. Endothelial dysfunction and non-alcoholic liver steatosis in hypertensive patients. Nutr Metab Cardiovasc Dis 2011;21(7):485–491. doi:10.1016/j.numecd.2009.11.015, PMID:20227260.
[104] Vlachopoulos C, Manesis E, Baou K, Papatheodoridis G, Koskinas J, Tiniakos D, *et al*. Increased arterial stiffness and impaired endothelial function in nonalcoholic fatty liver disease: a pilot study. Am J Hypertens 2010;23(11):1183–1189. doi:10.1038/ajh.2010.144, PMID:20634799.
[105] Musso G, Gambino R, Bo S, Uberti B, Biroli G, Pagano G, *et al*. Should nonalcoholic fatty liver disease be included in the definition of metabolic syndrome? A cross-sectional comparison with Adult Treatment Panel III criteria in nonobese nondiabetic subjects. Diabetes Care 2008;31(3):562–568. doi:10.2337/dc07-1526, PMID:18056890.
[106] Senturk O, Kocaman O, Hulagu S, Sahin T, Aygun C, Konduk T, *et al*. Endothelial dysfunction in Turkish patients with non-alcoholic fatty liver disease. Intern Med J 2008;38(3):183–189. doi:10.1111/j.1445-5994.2007.01481.x, PMID:17725609.
[107] Akyol G, Erdem O, Yilmaz G. Nonalcoholic fatty liver disease. Correlation with histology and viral hepatitis. Saudi Med J 2005;26(12):1904–1910. PMID:16380770.
[108] Villanova N, Moscatiello S, Ramilli S, Bugianesi E, Magalotti D, Vanni E, *et al*. Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. Hepatology 2005;42(2):473–480. doi:10.1002/hep.20781, PMID:15981216.
[109] Mazzella N, Ricciardi LM, Mazzotti A, Marchesini G. The role of medications for the management of patients with NAFLD. Clin Liver Dis 2014;18(1):73–89. doi:10.1016/j.cld.2013.09.005, PMID:24274866.
[110] Perazzo H, Dufour JF. The therapeutic landscape of non-alcoholic steatohepatitis. Liver Int 2017;37(5):634–647. doi:10.1111/liv.13270, PMID:27727520.
[111] Pasarín M, Abraldes JG, Liguori E, Kok B, La Mura V. Intrahepatic vascular changes in non-alcoholic fatty liver disease: Potential role of insulin-resistance and endothelial dysfunction. World J Gastroenterol 2017;23(37):6777–6787. doi:10.3748/wjg.v23.i37.6777, PMID:29085222.
[112] Ramadori P, Klag T, Malek NP, Heikenwalder M. Platelets in chronic liver disease, from bench to bedside. JHEP Rep 2019;1(6):448–459. doi:10.1016/j.jhepr.2019.10.001, PMID:32039397.
[113] Liu F, Zhou H, Cao L, Guo Z, Dong C, Yu L, *et al*. Risk of reduced platelet counts in patients with nonalcoholic fatty liver disease (NAFLD): a prospective cohort study. Lipids Health Dis 2018;17(1):221. doi:10.1186/s12944-018-0865-7, PMID:30227874.
[114] Shin WY, Jung DH, Shim JY, Lee HR. The association between non-alcoholic hepatic steatosis and mean platelet volume in an obese Korean population. Platelets 2011;22(6):442–446. doi:10.3109/09537104.2010.540049, PMID:21751850.
[115] Madan SA, John F, Pitchumoni CS. Nonalcoholic fatty liver disease and mean platelet volume: a systemic review and meta-analysis. J Clin Gastroenterol 2016;50(1):69–74. doi:10.1097/MCG.0000000000000340, PMID:25984978.
[116] Alkhouri N, Kistangari G, Campbell C, Lopez R, Zein NN, Feldstein AE. Mean platelet volume as a marker of increased cardiovascular risk in patients with nonalcoholic steatohepatitis. Hepatology 2012;55(1):331. doi:10.1002/hep.24721, PMID:21969091.
[117] Arslan N, Makay B. Mean platelet volume in obese adolescents with nonalcoholic fatty liver disease. J Pediatr Endocrinol Metab 2010;23(8):807–813. doi:10.1515/jpem.2010.130, PMID:21073123.
[118] Celikbilek M, Gürsoy S, Deniz K, Karaman A, Zararsiz G, Yurci A. Mean platelet volume in biopsy-proven non-alcoholic fatty liver disease. Platelets 2013;24(3):194–199. doi:10.3109/09537104.2012.688898, PMID:22646469.
[119] Santilli F, Blardi P, Scapellato C, Bocchia M, Guazzi G, Terzuoli L, *et al*. Decreased plasma endogenous soluble RAGE, and enhanced adipokine secretion, oxidative stress and platelet/coagulative activation identify non-alcoholic fatty liver disease among patients with familial combined hyperlipidemia and/or metabolic syndrome. Vascul Pharmacol 2015;72:16–24. doi:10.1016/j.vph.2015.04.004, PMID:26117210.
[120] Ferri F, Mischitelli M, Tozzi G, Messina E, Mignini I, Mazzuca S, *et al*. Reduced lysosomal acid lipase activity in blood and platelets is associated with non-alcoholic fatty liver disease. Clin Transl Gastroenterol 2020;11(2):e00116. doi:10.14309/ctg.0000000000000116, PMID:32463622.
[121] Malehmir M, Pfister D, Gallage S, Szydlowska M, Inverso D, Kotsiliti E, *et al*. Platelet GPIbα is a mediator and potential interventional target for NASH and subsequent liver cancer. Nat Med 2019;25(4):641–655. doi:10.1038/s41591-019-0379, PMID:30936549.
[122] McFadyen JD, Peter K. Platelet lipidomics and function: Joining the dots. Blood 2018;132:465–466. doi:10.1182/blood-2018-06-854950, PMID:30072414.
[123] Simon TG, Henson J, Osganian S, Masia R, Chan AT, Chung RT, *et al*. Daily aspirin use associated with reduced risk for fibrosis progression in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2019;17(13):2776–2784.e4. doi:10.1016/j.cgh.2019.04.061, PMID:31077838.
[124] Schwarzkopf K, Bojunga J, Rüschenbaum S, Martinez Y, Mücke M, Seeger F, *et al*. Use of antiplatelet agents is inversely associated with liver fibrosis in patients with cardiovascular disease. Hepatol Commun 2018;2(12):1601–1609. doi:10.1002/hep4.1254, PMID:30556044.
[125] Saremi Z, Rastgoo M, Mohammadifard M, Bijari B, Akbari E. Comparison of platelet number and function between nonalcoholic fatty liver disease and normal individuals. J Res Med Sci 2017;22:75. doi:10.4103/jrms.JRMS_711_16, PMID:28717372.
[126] Santilli F, Blardi P, Scapellato C, Bocchia M, Guazzi G, Terzuoli L, *et al*. Decreased plasma endogenous soluble RAGE, and enhanced adipokine secretion, oxidative stress and platelet/coagulative activation identify non-alcoholic fatty liver disease among patients with familial combined hyperlipidemia and/or metabolic syndrome. Vascul Pharmacol 2015;72:16–24. doi:10.1016/j.vph.2015.04.004, PMID:26117210.
[127] Kilciler G, Genc H, Tapan S, Ors F, Kara M, Karadurmus N, *et al*. Mean platelet volume and its relationship with carotid atherosclerosis in subjects with non-alcoholic fatty liver disease. Ups J Med Sci 2010;115(4):253–259. doi:10.3109/03009734.2010.500062, PMID:20731535.
[128] Ozhan H, Aydin M, Yazici M, Yazgan O, Basar C, Gungor A. Mean platelet volume in patients with non-alcoholic fatty liver disease. Platelets

2010;21(1):29–32. doi:10.3109/09537100903391023, PMID:19947902.
[129] Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, *et al*. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44(4):865–873. doi:10.1002/hep.21327, PMID:17006923.
[130] Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. J Hepatol 2008;49(4):608–612. doi:10.1016/j.jhep.2008.06.018, PMID:18682312.
[131] Targher G, Arcaro G. Non-alcoholic fatty liver disease and increased risk of cardiovascular disease. Atherosclerosis 2007;191(2):235–240. doi:10.1016/j.atherosclerosis.2006.08.021, PMID:169709551.
[132] Ekstedt M, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S, *et al*. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology 2015;61(5):1547–1554. doi:10.1002/hep.27368, PMID:25125077.
[133] Zeb I, Li D, Budoff MJ, Katz R, Lloyd-Jones D, Agatston A, *et al*. Nonalcoholic fatty liver disease and incident cardiac events: the multi-ethnic study of atherosclerosis. J Am Coll Cardiol 2016;67(16):1965–1966. doi:10.1016/j.jacc.2016.01.070, PMID:27102512.
[134] Stine JG, Niccum BA, Zimmet AN, Intagliata N, Caldwell SH, Argo CK, *et al*. Increased risk of venous thromboembolism in hospitalized patients with cirrhosis due to non-alcoholic steatohepatitis. Clin Transl Gastroenterol 2018;9(3):140. doi:10.1038/s41424-018-0002-y, PMID:29511162.
[135] Stine JG, Argo CK, Pelletier SJ, Maluf DG, Caldwell SH, Northup PG. Advanced non-alcoholic steatohepatitis cirrhosis: A high-risk population for pre-liver transplant portal vein thrombosis. World J Hepatol 2017;9(3):139–146. doi:10.4254/wjh.v9.i3.139, PMID:28217250.
[136] Stine JG, Intagliata N, Northup PG, Caldwell SH. Nonalcoholic fatty liver disease, portal vein thrombosis and coagulation: More questions than answers? Transplantation 2017;101(8):e281–e282. doi:10.1097/TP.0000000000001807, PMID:28471888.
[137] Stine JG, Wang J, Shah PM, Argo CK, Intagliata N, Uflacker A, *et al*. Decreased portal vein velocity is predictive of the development of portal vein thrombosis: A matched case-control study. Liver Int 2018;38(1):94–101. doi:10.1111/liv.13500, PMID:28632958.
[138] Lin H, Bai Z, Guo X, Qi X. Association between nonalcoholic fatty liver disease and portal vein thrombosis: a systematic review and meta-analysis. Eur J Gastroenterol Hepatol 2020;32(10):1405–1406. doi:10.1097/MEG.0000000000001689, PMID:32858662.
[139] Abdel-Razik A, Mousa N, Abdelsalam M, Wahba NM, Helmy EM, Tawfi AM, *et al*. De novo portal vein thrombosis in non-cirrhotic non-alcoholic fatty liver disease: A 9 year prospective cohort study. Front Med 2021;8:650818. doi:10.3389/fmed.2021.650818, PMID:33996858.
[140] Wanless IR, Wong F, Blendis LM, Greig P, Heathcote EJ, Levy G. Hepatic and portal vein thrombosis in cirrhosis: possible role in development of parenchymal extinction and portal hypertension. Hepatology 1995;21(5):1238–1247. PMID:7737629.
[141] Kassel KM, Owens AP 3rd, Rockwell CE, Sullivan BP, Wang R, Tawfik O, *et al*. Protease-activated receptor 1 and hematopoietic cell tissue factor are required for hepatic steatosis in mice fed a Western diet. Am J Pathol 2011;179(5):2278–2289. doi:10.1016/j.ajpath.2011.07.015, PMID:21907177.
[142] Owens AP 3rd, Passam FH, Antoniak S, Marshall SM, McDaniel AL, Rudel L, *et al*. Monocyte tissue factor-dependent activation of coagulation in hypercholesterolemic mice and monkeys is inhibited by simvastatin. J Clin Invest 2012;122(2):558–568. doi:10.1172/JCI58969, PMID:22214850.
[143] Nault R, Fader KA, Kopec AK, Harkema JR, Zacharewski TR, Luyendyk JP. From the cover: Coagulation-driven hepatic fibrosis requires protease activated receptor-1 (PAR-1) in a mouse model of TCDD-elicited steatohepatitis. Toxicol Sci 2016;154(2):381–391. doi:10.1093/toxsci/kfw175, PMID:27613713.
[144] Borensztajn K, von der Thüsen JH, Peppelenbosch MP, Spek CA. The coagulation factor Xa/protease activated receptor-2 axis in the progression of liver fibrosis: a multifaceted paradigm. J Cell Mol Med 2010;14(1-2):143–153. doi:10.1111/j.1582-4934.2009.00980.x, PMID:19968736.
[145] Spooner MH, Jump DB. Omega-3 fatty acids and nonalcoholic fatty liver disease in adults and children: where do we stand? Curr Opin Clin Nutr Metab Care 2019;22(2):103–110. doi:10.1097/MCO.0000000000000539, PMID:30601174.
[146] Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, *et al*. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018;67(1):328–357. doi:10.1002/hep.29367, PMID:28714183.
[147] Adili R, Hawley M, Holinstat M. Regulation of platelet function and thrombosis by omega-3 and omega-6 polyunsaturated fatty acids. Prostaglandins Other Lipid Mediat 2018;139:10–18. doi:10.1016/j.prostaglandins.2018.09.005, PMID:30266534.
[148] Calder PC. The role of marine omega-3 (n-3) fatty acids in inflammatory processes, atherosclerosis and plaque stability. Mol Nutr Food Res 2012;56(7):1073–1080. doi:10.1002/mnfr.201100710, PMID:22760980.
[149] Kothari S, Dhami-Shah H, Shah SR. Antidiabetic Drugs and Statins in Nonalcoholic Fatty Liver Disease. J Clin Exp Hepatol 2019;9(6):723–730. doi:10.1016/j.jceh.2019.06.003, PMID:31889754.
[150] Janicko M, Drazilova S, Pella D, Fedacko J, Jarcuska P. Pleiotropic effects of statins in the diseases of the liver. World J Gastroenterol 2016;22(27):6201–6213. doi:10.3748/wjg.v22.i27.6201, PMID:27468210.
[151] Liao JK, Laufs U. Pleiotropic effects of statins. Annu Rev Pharmacol Toxicol 2005;45:89–118. doi:10.1146/annurev.pharmtox.45.120403.095748, PMID:15822172.
[152] Pella D, Rybar R, Mechirova V. Pleiotropic effcts of statins. Acta Cardiologica Sinica 2005;21:190–198.
[153] Athyros VG, Tziomalos K, Gossios TD, Griva T, Anagnostis P, Kargiotis K, *et al*. Safety and efficacy of long-term statin treatment for cardiovascular events in patients with coronary heart disease and abnormal liver tests in the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) Study: a post-hoc analysis. Lancet 2010;376(9756):1916–1922. doi:10.1016/S0140-6736(10)61272-X, PMID:21109302.
[154] Anstee QM, Wright M, Goldin R, Thursz MR. Parenchymal extinction: coagulation and hepatic fibrogenesis. Clin Liver Dis 2009;13(1):117–126. doi:10.1016/j.cld.2008.09.013, PMID:19150316.
[155] Tripodi A, Pellegatta F, Chantarangkul V, Grigore L, Garlaschelli K, Baragetti A, *et al*. Statins decrease thrombin generation in patients with hypercholesterolemia. Eur J Intern Med 2014;25(5):449–451. doi:10.1016/j.ejim.2014.03.016, PMID:24784951.
[156] Undas A, Brummel-Ziedins KE, Mann KG. Anticoagulant effects of statins and their clinical implications. Thromb Haemost 2014;111(3):392–400. doi:10.1160/TH13-08-0720, PMID:24285296.
[157] Kostapanos MS, Kei A, Elisaf MS. Current role of fenofibrate in the prevention and management of non-alcoholic fatty liver disease. World J Hepatol 2013;5(9):470–478. doi:10.4254/wjh.v5.i9.470, PMID:24073298.
[158] Lalloyer F, Wouters K, Baron M, Caron S, Vallez E, Vanhoutte J, *et al*. Peroxisome proliferator-activated receptor-alpha gene level differently affects lipid metabolism and inflammation in apolipoprotein E2 knock-in mice. Arterioscler Thromb Vasc Biol 2011;31(7):1573–1579. doi:10.1161/ATVBAHA.110.220525, PMID:21474829.
[159] Liu Q, Liu S, Chen L, Zhao Z, Du S, Dong Q, *et al*. Role and effective therapeutic target of gut microbiota in NAFLD/NASH. Exp Ther Med 2019;18(3):1935–1944. doi:10.3892/etm.2019.7781, PMID:31410156.
[160] Tsuji Y, Kaji K, Kitade M, Kaya D, Kitagawa K, Ozutsumi T, *et al*. Bile acid sequestrant, sevelamer ameliorates hepatic fibrosis with reduced overload of endogenous lipopolysaccharide in experimental nonalcoholic steatohepatitis. Microorganisms 2020;8(6):925. doi:10.3390/microorganisms8060925, PMID:32575352.
[161] Rastogi A. Sevelamer revisited: pleiotropic effects on endothelial and cardiovascular risk factors in chronic kidney disease and end-stage renal disease. Ther Adv Cardiovasc Dis 2013;7(6):322–342. doi:10.1177/1753944713513061, PMID:24327730.
[162] Villa E, Cammà C, Marietta M, Luongo M, Critelli R, Colopi S, *et al*. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. Gastroenterology 2012;143:1253–1260.e4. doi:10.1053/j.gastro.2012.07.018, PMID:22819864.