Hemostasis and anticoagulant therapy in liver diseases
Potze, Wilma
Vascular disease in patients with nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is increasingly being diagnosed and is considered to be the most frequent chronic liver disorder in Western countries. It represents a histopathological spectrum ranging from simple hepatic steatosis to steatohepatitis and finally cirrhosis. NAFLD is considered as the hepatic manifestation of the metabolic syndrome and is associated with increased mortality. Increasing evidence now suggests that NAFLD is also associated with higher cardiovascular disease (CVD) morbidity and mortality independent of conventional cardio metabolic risk factors (such as obesity, insulin resistance, and diabetes mellitus). The exact mechanisms linking NAFLD to increased CVD risk are still incompletely understood and likely reflect multiple coexisting pathways. Recent evidence suggests a contributive effect of an altered hemostasis in patients with NAFLD. For example, patients with NAFLD have higher levels of pro-thrombotic factors (e.g., von Willebrand factor, fibrinogen, factor VII activity, and plasminogen activator inhibitor-1), which correlate with underlying histological severity of the disease. The current review focuses on these hemostatic abnormalities in NAFLD and the link with increased CVD risk.
Introduction
Nonalcoholic fatty liver disease (NAFLD) is the most common cause of abnormally elevated aminotransferases [1,2] with an estimated prevalence ranging from 6.3 to 33% with a median of 20% in the general population [2]. Its prevalence increases from 70 to 90% among those with diabetes or obesity [1,2]. NAFLD is characterized by fat accumulation in the liver in the absence of excessive alcohol consumption (less than 20 g/day in women and less than 40 g/day in men) and exclusion of other secondary causes of hepatic steatosis [3–6]. NAFLD represents a histopathological spectrum ranging from simple hepatic steatosis to steatohepatitis (NASH), characterized by necroinflammatory changes which increase the risk for progression to advanced fibrosis and cirrhosis [6–8]. NAFLD is considered the hepatic manifestation of the metabolic syndrome and is associated with increased mortality, particularly cardiovascular disease (CVD)-related mortality [7–9]. The metabolic syndrome is a cluster of metabolic abnormalities that predicts the risk of developing diabetes and CVD better than any of its individual components [10]. The metabolic syndrome predicts the development of NAFLD, and the risk of having NAFLD increases significantly with the addition of each of the component of the metabolic syndrome [11,12].

Cardiovascular Disease and Nonalcoholic Fatty Liver Disease
Patients with NAFLD have multiple traditional and nontraditional risk factors for CVD, and CVD-related mortality is the leading cause of death in those with NAFLD [7,8,13] Although NAFLD is commonly seen with other features of the metabolic syndrome, the increased CVD risk in NAFLD is independent of obesity, diabetes, and other features of the metabolic syndrome [14,15]. Accumulating evidence implicates NAFLD as a risk factor not only for CVD events but also for subclinical and premature CVD [16] Presence of NAFLD is associated with increased carotid artery intimal medial thickness (IMT), a validated marker of subclinical atherosclerosis [14]. These findings were corroborated in a larger meta-analysis that clearly showed that the presence of NAFLD was associated with carotid artery IMT and prevalence of carotid artery atherosclerotic disease [17]. The IMT was the greatest in those with steatohepatitis, then in those with simple hepatic steatosis, and the lowest in healthy individuals [14]. In addition, the association between NAFLD and the increased risk of coronary artery disease (as measured by coronary artery calcium score or CAC) is independent of conventional risk factors, metabolic syndrome features, insulin resistance, and even preexisting CVD [18].

The risk of clinically evident CVD is also much higher in those with NAFLD and is independent of classical CVD risk factors, features of metabolic syndrome, and insulin resistance [15,19]. In addition, the presence of NAFLD was associated with lower remodeling lesions or lipid core plaques of coronary arteries, thereby implicating NAFLD as a risk factor for vulnerable coronary artery plaques [20]. In a community-based cohort those with NAFLD had a much higher 10-year FraminghamRisk Score (FRS) than matched controls [21]. In fact, in a recent meta-analysis, presence of NAFLD was associated with increased overall mortality (odds ratio [OR]: 1.57; confidence interval [CI]: 1.18–2.10) deriving from liver related and cardiovascular-related mortality [22]. The exact mechanisms linking NAFLD to increased CVD risk are incompletely understood and likely reflect multiple coexisting pathways, one of which is altered hemostasis in those with NAFLD. The current review focuses on these hemostatic abnormalities in NAFLD.
Hemostasis in Nonalcoholic Fatty Liver Disease

A link between NAFLD and CVD is their close association with pro-inflammatory markers, such as high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), tumor necrosis factor-a (TNF-a), and other acute phase reactants [23-26]. In addition to these pro-inflammatory markers, NASH is also considered to be associated with a pro-thrombotic state and the studies investigating this association are summarized in Table 1.

Nonalcoholic Fatty Liver Disease and Platelets

It is well known that platelet activation is increased in the metabolic syndrome and obesity [46-51], likely reflecting the altered biophysical state of the platelet membrane component in these patients. Platelets of these individuals have an enhanced expression of the platelet surface of adhesive receptors, such as P-selectin, αIIbβ3, and the glycoprotein (GP)Ib/IX/V complex. The increased expression of the latter two receptors most probably causes more frequent episodes of platelet activation and degranulation [52]. Patients with metabolic syndrome also have concurrent endothelial dysfunction which may trigger platelet activation. Finally, hypertriglyceridemia, a key clinical feature of NASH, is a known trigger for platelet activation and may contribute to the underlying thrombotic risk [53,54]. Studies have also shown an increase in mean platelet volume, a reliable indicator of platelet activation, in patients with NAFLD [27-29]. In fact, in a cohort of 100 consecutive patients undergoing liver biopsy for NAFLD, the mean platelet volume increased in a stepwise fashion from those with no steatosis to those with simple steatosis to those with NASH [30]. Furthermore, in a group of 44 NASH patients, an increase in platelet count was observed [55]. However, the increase in platelet count is followed by a steady decrease in platelet count when the disease progresses to fibrosis/cirrhosis [56]. Finally, von Willebrand factor (VWF), a mediator of platelet adhesion and aggregation, was found to be significantly elevated in patients with NAFLD and this may be a consequence of endothelial injury [31,34,43].

Nonalcoholic Fatty Liver Disease and Hemostatic Abnormalities

In addition to the increased platelet activation, patients with NAFLD also have higher levels of pro-thrombotic factors (e.g., VWF, fibrinogen, factor VII activity, and plasminogen activator inhibitor-1 [PAI-1]) and this directly correlates with underlying histology [57-59]. For example, Kotronen et al observed that the plasma levels of factors VIII, IX, XI, and XII were increased in 54 patients with NAFLD compared with 44 controls [32]. The associations between NAFLD and these coagulation factors were independent of age, gender, and body mass index, suggesting that the presence of hepatic steatosis independently contributes to the thrombosis risk. More recently, Verrijken et al found significantly higher levels of PAI-1 in patients with NASH than controls and this association correlated with underlying histological parameters [31]. However, in patients with NAFLD plasma levels of fibrinogen, factor VIII, VWF, and antithrombin did not correlate with liver histology, but did correlate with features of the metabolic syndrome. Elevated levels of PAI-1 might, therefore, partly explain the increased cardiovascular risk seen in patients with NAFLD. Indeed, increased levels of PAI-1, with resultant reduction in fibrinolysis, have been extensively reported as a risk factor for thrombosis and cardiovascular events [60], although some studies suggest PAI-1 merely to be a reflection of general cardiovascular risk factors [61]. The liver is the site of synthesis for a majority of pro-coagulant proteins. Several studies have now documented an increased intrahepatic expression of these proteins in patients with NAFLD [35,36], suggesting that this increase may be because of increased hepatic synthesis [58,59]. These findings are further
corroborated by previous studies that have shown dysregulation of several candidate genes responsible for accelerated atherogenesis in the liver of those with NASH [62–64]. Despite the initial findings between NAFLD and hemostatic abnormalities, the data should be interpreted with caution as most of these studies are limited by their relatively small sample size and cross-sectional design. In addition, patients with cirrhosis were excluded from most of these studies, so it is not clear how progression to cirrhosis impacts these parameters. Nevertheless, some evidence does suggest that patients with NASH-related compensated cirrhosis have an increased risk of atherothrombotic events [65]. In a prospective study, it was demonstrated that, while patients with compensated cirrhosis because of NASH have a lower overall mortality rate than patients with hepatitis C virus-associated cirrhosis, cardiovascular mortality is increased in patients with NASH-related cirrhosis than those with hepatitis C virus-associated cirrhosis [66].

To date, only one recent study has examined the coagulation status in patients with NASH-related cirrhosis [33]. The authors observed a pro-coagulant imbalance characterized by increased levels of factor VIII and a reduction in protein C in patients with NAFLD and these differences were more pronounced in those with NASH and NASH-cirrhosis than those with simple steatosis. However, they found no differences in alcoholic/viral-related cirrhosis, in which a pro-coagulant imbalance was also observed, as has been previously noted [67-69]. This observation, therefore, does not explain the increased risk of cardiovascular disease in NASH cirrhosis compared with alcoholic/viral cirrhosis. Furthermore, the authors only show the endogenous thrombin potential (ETP) ratio, which represents the resistance of the plasma to the anticoagulant action of thrombomodulin, and do not depict the original thrombin-generation data. More specifically, they do not compare original thrombin-generation data, for example, ETP in the presence of thrombomodulin between patients with NAFLD and controls. If the original ETP values are indeed significantly increased in patients with NAFLD compared with controls, this implies a hypercoagulable status in plasma of patients with NAFLD. Thus, to confirm these findings, further studies are needed to investigate the hemostatic abnormalities in patients with advanced NAFLD.

<table>
<thead>
<tr>
<th>Primary hemostasis: Platelet dysfunction</th>
<th>Secondary hemostasis: Hypercoagulability</th>
<th>Tertiary hemostasis: Hypofibrinolysis</th>
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<tbody>
<tr>
<td>Elevated levels of VWF [31,34,43]</td>
<td>High plasma levels of factor VII clotting activity [37]</td>
<td>Low levels of TAFI antigen [38]</td>
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<tr>
<td></td>
<td>Elevated levels of fibrinogen [24,31,34,44]</td>
<td>Low levels of t-PA [37]</td>
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<td>Low levels of antithrombin III [31]</td>
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<td>Levels of protein C either increased [44,45] or decreased [33]</td>
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**Table 1.** Summary of studies on the changes in all phases of the hemostasis in patients with nonalcoholic fatty liver disease.

**Risk of Venous Thrombosis in Nonalcoholic Fatty Liver Disease**

Recent epidemiological studies have challenged the notion that patients with a chronic liver disease are auto-anticoagulated, and, hence, are protected against venous thrombosis (including deep vein thrombosis [DVT] and pulmonary embolism) [70–74]. Rather, there is evolving evidence to suggest a higher propensity for developing venous thrombosis in patients with a liver disease [75–77].

While there are multiple case-control studies that have shown an increase in circulating levels of various pro-thrombotic proteins in plasma of patients with NAFLD (see previous section and Table 1), there is currently little published research that assessed the association between NAFLD/NASH and the risk of venous thrombosis. Although there is lack of data in NAFLD patients, there are numerous studies that implicate obesity, a common feature of NAFLD, as a risk factor for the development of venous thromboembolism (VTE) [78–80]. In the limited data available, an independent association between NAFLD and idiopathic VTE was noted and the prevalence of NAFLD increased nearly threefold in those with VTE [81]. A possible cause of the increased risk of venous thrombosis in NAFLD patients might be the elevated levels of PAI-1 resulting in hypofibrinolysis in these patients. However, there is currently little known about the incidence of venous thrombosis in NASH-related cirrhosis. In addition, a recent retrospective study reported an increased prevalence of portal vein thrombosis (PVT) in patients with NASH related cirrhosis, and the presence of NASH was an independent risk factor for the diagnosis of PVT [82]. Because of the limited number of studies, these data should be interpreted with caution and well-designed prospective studies are needed to further explore the relationship between NAFLD/NASH fibrosis stage and the risk of venous thrombosis.

**Role of Hypercoagulability in Liver Fibrogenesis**

The progression from chronic liver injury to advanced hepatic fibrosis or cirrhosis is a complex process, mediated through the interaction between genetic and environmental factors. Recent evidence suggests a role for the activity of the coagulation cascade in hepatic fibrogenesis [75,83]. For example, epidemiological evidence has shown an association between congenital coagulation abnormalities (e.g., increased expression of factor VIII, protein C deficiency, and factor V Leiden mutation) and accelerated progression to cirrhosis in patients with a chronic hepatitis C infection [84–87]. Furthermore, Assy et al observed a correlation between the presence of thrombotic risk factors and the extent of hepatic fibrosis in patients with NASH [45] Based on recent evidence, there may be two hypotheses that could explain the involvement of the coagulation cascade in the rate of liver fibrogenesis: tissue ischemia because of intrahepatic thrombi (also referred to as parenchymal extinction) and the activation of stellate cells by coagulation proteases [83].

Accumulating animal data suggest that antithrombotic treatment may retard the progression of fibrosis by inhibiting the coagulation or the platelet function [88–92]. Fujita et al have shown that antiplatelet therapy reduces hepatic steatosis, inflammation, and hepatic fibrosis in rats [93]. Also, anticoagulant therapy decreased fatty liver disease in an experimental animal model [94]. If antithrombotic treatment truly prevents progression of disease and substantially delays decompensation, the need for liver transplantation, or death, this will profoundly impact the clinical management of patients with a chronic liver disease. Nevertheless, several recent experimental animal studies suggest that platelets and fibrin
may, on the contrary, have beneficial effects on liver injury [95–98]. Future clinical studies on the benefits and safety of antithrombotic treatment in different patient groups are, therefore, required before this may be considered in routine practice. NAFLD might be a reasonable disease state to further investigate these treatment strategies in human trials, because of the suggested hypercoagulable state and the relative slow course of the disease. A major limitation to evaluating coagulation defects clinically is the lack of readily available laboratory tests to measure hypercoagulability in patients with a chronic liver disease.

**Conclusion**

NAFLD, the most common cause of liver disease globally, is associated with higher CVD morbidity and mortality. Multiple processes probably contribute to this increased risk of CVD, including abnormal glucose, fatty acid, and lipoprotein metabolism, chronic inflammation, increased oxidative stress, deranged adipokine function, endothelial dysfunction, atherosclerosis, and cardiac lipotoxicity. Several studies have also suggested a role for the presence of a hypercoagulable state in the increased risk of CVD in patients with NAFLD. The present evidence strongly highlights the importance of evaluating the risk of CVD and related pro-inflammatory/pro-thrombotic state in patients with NAFLD. Clinicians should recognize the increased risk of CVD among patients with NAFLD, which warrants evaluation and treatment as much as the liver disease itself.
References


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89. Wilma Potze, Proefschrift 2017_170x240_Binnen_FINAL.indd 93
Hemostasis and Anticoagulant Therapy in Liver Diseases