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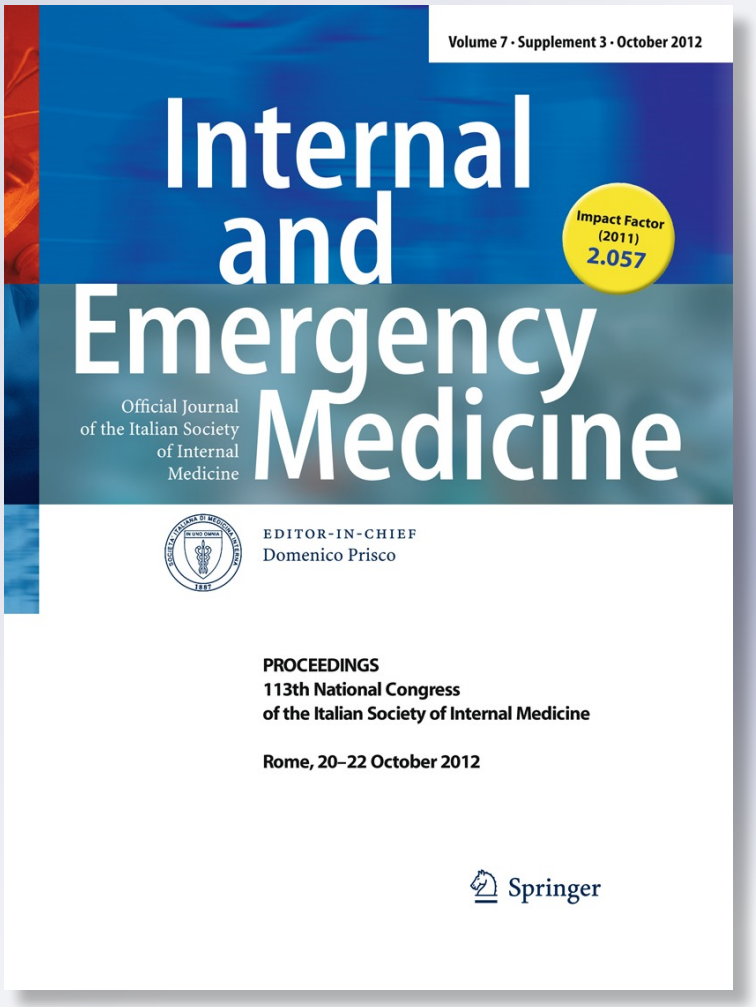

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Non-alcoholic fatty liver disease and cardiovascular disease: epidemiological, clinical and pathophysiological evidences

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Abstract Non-alcoholic fatty liver disease is recognized as the most common and emerging chronic liver disease in western countries. The disease has been traditionally interpreted as a possibly progressing condition to liver fibrosis and cirrhosis. However, recently, a large number of publications have demonstrated that people with non-alcoholic fatty liver have an increased chance of developing cardiovascular diseases, which represent the major causes of death in this setting. This association is mainly explained by the atherogenic profile of the metabolic syndrome a condition frequently associated with fatty liver, which may represent its hepatic component. Some studies have also shown an association independent of traditional risk factors or of the clinical features of the metabolic syndrome. In this setting, cardiovascular disease seems to be the consequence of low-grade chronic inflammation and increased oxidative stress. Most studies did not differentiate cardiovascular risk between simple steatosis and non-alcoholic steatohepatitis, although the latter seems to be at higher cardiovascular risk. Few studies have investigated the direct correlation between hepatic inflammation and atherosclerosis. Genetic studies will probably improve the interpretation of the increased cardiovascular risk in patients with fatty liver and no metabolic syndrome.

Keywords Non-alcoholic fatty liver disease · Non-alcoholic steatohepatitis · Cardiovascular disease · Metabolic syndrome

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a condition characterized by fatty deposition in the liver including a wide spectrum of histologic alterations ranging from simple hepatic steatosis, having usually a benign clinical course, to non-alcoholic steatohepatitis (NASH). NASH, which is characterized by inflammation, can progress to fibrosis and cirrhosis or even hepatocellular carcinoma [1, 2].

NAFLD is now recognized as the most common and emerging chronic liver disease in western countries and is now spreading rapidly to other parts of the world [3–5]. NASH is the third most common indication for liver transplantation in the United States and is projected to eventually overtake the hepatitis C virus and alcoholic liver disease as the leading cause of liver transplant [6].

The pathogenesis of non-alcoholic and virus-negative liver steatosis and NASH appears multifactorial and many mechanisms have been proposed as causes of fatty liver infiltration. The association of steatosis with a number of different clinical conditions has been suggested. It frequently occurs with the features of the metabolic syndrome (MetS) and it has been suggested that it may represent its hepatic manifestation. Common metabolic diseases—hyperlipidemia, type 2 diabetes, insulin resistance (IR) and central obesity—have been associated with both benign liver steatosis and progressive NASH.

According to current opinion, the pathogenesis of NAFLD is based on a "two hits hypothesis" [7, 8]. The "first hit" involves hepatic triglyceride accumulation (i.e., simple steatosis) mainly due to IR thus increasing vulnerability to further insults. The "second hit" is mainly due to chronic oxidative stress, which leads to depletion of the natural antioxidants and results in excess reactive oxidative

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species within the hepatocyte. It includes lipid peroxidation, liver damage, mitochondrial dysfunction, and inflammation, which in turn may progress to steatohepatitis and fibrosis. Lipid peroxidation promotes stellate cell proliferation, which in turn is primarily responsible for fibrinogenesis.

Liver biopsy represents the best diagnostic test to stage hepatic steatosis, inflammation, and fibrosis. NAFLD histology includes simple steatosis or in more severe cases a combination of steatosis, inflammatory cell infiltration, hepatocyte ballooning and spotty necrosis [9]. However, most of patients with liver steatosis can be well managed without a need for liver biopsy, which cannot be performed at large in patients with no significant or trivial liver disease, mainly for ethical reasons. Liver ultrasonographic (US) scan, although not sufficiently sensitive to detect liver inflammation and fibrosis, has a good correlation with the histologic finding of fatty infiltration and international guidelines have been proposed for the US evaluation of the different degree of steatosis [10, 11].

NAFLD has been traditionally interpreted as a condition, which may progress to liver related complications such as cirrhosis, liver cancer, and liver mortality. In particular, liver fibrosis predicts disease progression and the risk for hepatocellular carcinoma. However, most people with NAFLD in the absence of significant hepatic fibrosis do not develop serious liver problems. Conversely, people with NAFLD have an increased chance of developing cardiovascular diseases, such as myocardial infarction and stroke, which represent the major causes of death in this setting. Indeed, cardiovascular disease is the single most important cause of morbidity and mortality in this patient population. Therefore, it appears that the increased mortality of patients is primarily a result of cardiovascular diseases and, to a lesser extent, to liver related diseases. Indeed, many epidemiological, clinical, and pathophysiological observations support a strong association between NAFLD and increased cardiovascular risk, which in some studies was found to be independent of traditional risk factors and aspects of the MetS [12, 13]. Moreover, patients with NASH seem to be at higher risk for atherosclerosis than patients with simple steatosis as a consequence of chronic inflammation and oxidative stress. Finally, an increased risk of developing type 2 diabetes mellitus has also been demonstrated among NAFLD patients [14]. Therefore, based on the above considerations, we may perhaps consider patients with NAFLD at increased global cardiometabolic risk, although the precise mechanisms by which NAFLD contributes to cardiovascular disease (CVD) and diabetes are still the subject of ongoing research. Several studies have examined the relation of NAFLD with functional and structural alterations of the arteries and with metabolic alterations, inflammatory markers and fat-produced hormones, which have been linked with increased cardiovascular risk. The awareness of NAFLD as a cardiovascular risk factor is still emerging and over 450 articles have been published over the last decade. In this review, we discuss the epidemiological, clinical, and physiopathological evidence of an association between NAFLD and cardiovascular diseases.

Epidemiological association of NAFLD with CV diseases

Epidemiological studies indicate that NAFLD is associated with an increased risk for cardiovascular disease. Steatosis seems to be associated with an increased prevalence and incidence of cardiovascular disease and cardiovascular mortality.

However, most of the data are derived from studies with small numbers of subjects where NAFLD was diagnosed by ultrasounds or by surrogate markers like liver enzymes, rather than liver biopsy. Moreover, few studies, which differentiated between simple steatosis and NASH, have investigated the direct correlation between hepatic inflammation and atherosclerosis.

In a large study performed in the US population NAFLD, based on ultrasound analysis, was associated independently with an increased risk of CVD. However, NAFLD did not increase cardiovascular mortality over a 14-year period [15]. In a prospective study of apparently healthy Japanese men and women where NAFLD was diagnosed by abdominal ultrasonography, the incidence of cardiovascular disease was higher in 231 subjects with NAFLD at baseline than in 990 subjects without NAFLD. Multivariate analyses indicated that NAFLD was a predictor of CVD independent of MetS and conventional risk factors [16].

Other studies investigated the association between liver enzymes, surrogate markers of steatosis, with cardiovascular diseases. In a meta-analysis with data pooled from 10 studies 1 U/L higher GGT (on a log scale) was associated with a 20 % increase in the risk of coronary heart disease (CHD), a 54 % increase in the risk of stroke, and a 34 % increase in the risk of CHD and stroke combined [17]. Conversely, the association of ALT with CVD event risk appears somewhat weaker and current evidence from the Framingham Offspring Study goes against an independent association of ALT with CVD events [18]. However, in a cross-sectional analysis comparing participants in the third National Health and Nutrition Examination Survey in US, it was noted that individuals with elevated ALT had not only NAFLD but also an increased calculated risk of coronary heart disease, as estimated using the Framingham risk score [19].

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A recent study of unselected patients with type 2 diabetes mellitus reported that the prevalence of cardiovascular, cerebrovascular, and peripheral vascular disease was significantly greater in those with NAFLD on ultrasound than in those without, and independent of the individual components of the metabolic syndrome [20]. However, none of the liver enzymes was independently associated with prevalent CVD after further adjustment for the metabolic syndrome and/or ultrasound-diagnosed NAFLD.

Few prospective studies have been based on gold standard liver biopsy-diagnosed NAFLD. In a Swedish cohort study, 129 consecutively enrolled patients diagnosed with biopsy proven NAFLD were revaluated and survival and causes of death were compared with a matched reference population. Mortality was not increased in patients with steatosis, while survival of patients with NASH was reduced. Most NAFLD patients died from cardiovascular diseases and many developed diabetes or impaired glucose tolerance [21].

Recently, the long-term prognosis for individuals with NAFLD and NASH was examined in a further Swedish cohort of subjects with elevated serum levels of aminotransaminases who underwent liver biopsy. During the follow-up period, subjects with simple steatosis exhibited a 55 % increased mortality and those with NASH a 86 %, compared with the total Swedish population, adjusted for sex, age, and calendar period. Subjects more often died from cardiovascular diseases [22].

Furthermore, in a study by Wong et al. [23] performed in 612 consecutive patients who underwent coronary angiogram and had ultrasound screening for fatty liver, $\geq$50 % stenosis in at least one coronary artery was present in 84.6 % of those with fatty liver and 64.1 % of those without, which confirmed a strong association between fatty liver and CHD after adjustment for many demographic and metabolic factors. However, in this study, fatty liver could not predict cardiovascular mortality and morbidity in patients with established CHD. Finally, two studies using multislice computed tomography showed that patients with NAFLD, even without MetS, have more vulnerable coronary soft plaques than healthy controls [24, 25].

NAFLD and surrogate markers of arteriosclerosis

Brachial artery flow-mediated dilation (FMD) is a test used for clinical purposes to assess the existence of arterial dysfunction, a hallmark of early stage, systemic atherosclerosis. [26]. Endothelial dysfunction has been observed in patients with NAFLD compared to control groups and an independent relationship between these two factors has been demonstrated [27, 28].

Furthermore, [29] proved a relationship between severity of NAFLD and endothelial disfunction, showing a lower FMD in biopsy-proved NASH compared with simple steatosis. Recently, in a large study performed in obese children, compared to normal weight controls and children without liver involvement, those with ultrasound-diagnosed NAFLD and elevated alanine aminotransferase demonstrated significantly impaired FMD after controlling for MetS and Tunner stage [30].

Carotid intima-media thickness (IMT) and carotid plaque ultrasound measurement are common screening tools for systemic atherosclerosis in asymptomatic subjects. NAFLD patients have increased subclinical atherosclerosis compared with non-steatosic individuals, as shown by increased carotid wall intimal thickness and increased numbers of atherosclerotic plaques.

In a systematic review, [31] remarked a strong association between IMT and NAFLD with a 13 % IMT increase in patients with hepatic steatosis in comparison with individuals without it. Similar findings have been also obtained in pediatric populations [32]. However, there are conflicting data in defining whether increased IMT is a consequence of the presence of MetS or if NAFLD represents an independent predictor. In Völzke's study [33], after an adjustment for age and gender as well as for possible confounders and atherogenic risk factors, differences between patients and control groups were not significant. In Kim's study [34], the independent association between NAFLD and IMT was limited to subjects with MetS or multiple metabolic abnormalities and authors suggest that NAFLD can be a marker of more severe metabolic disorders. Instead, several studies showed NAFLD as independent predictor of abnormal IMT [35–37]. Furthermore, Mohammadi et al. [38] observed an increased IMT in NAFLD patients without metabolic disorders.

Also in defining of relationship between IMT and NAFLD severity there are conflicting data. In one of the three major studies, in which NAFLD severity was defined by liver biopsy, no difference between NASH and simple steatosis was reported [27]. Negative findings were also reported in a sample of obese children and adolescents where no association was reported between histological severity and IMT in children with NAFLD [39].

Conversely, a close relation between liver histology and IMT was observed in other two studies [35, 36]. In particular, Brea et al. [35] defined a progressive IMT increase from control subjects without MetS, to controls with MetS, to NAFLD patients without MetS and those with MetS.

Many studies showed an increased prevalence of carotid plaques in patients with NAFLD compared with control individuals [33, 35, 37, 40]. In a systematic review, relative risk for carotid atherosclerosis was almost twice as high in patients with NAFLD [31].

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Finally, a study carried out in Japanese university students has shown that the brachial-ankle pulse wave velocity in male subjects was significantly higher in the obese than in the overweight group, and higher in those with than in those without NAFLD [41].

NAFLD and markers of systemic inflammation and oxidative stress

Hepatic steatosis may be actively involved in the pathogenesis of atherosclerosis, which includes a release of pro-atherogenic factors from the liver promoting subclinical inflammation, procoagulant factors and increased oxidative stress [42, 43]. Patients with NAFLD have higher levels of high-sensitivity C-reactive protein, TNF-α, plasma plasminogen activator inhibitor-1 and oxidized LDL and lower levels of adiponectin than controls [42, 44, 45].

NAFLD is associated with inflammatory markers and fat-produced hormones, which have been linked with increased cardiovascular risk. In a recent study, low adiponectin levels have also been associated with increased aminotransferase and gamma-GT levels, suggesting a role in liver cell integrity [46]. Inflammatory cytokines tend to be higher in patients with NASH compared with patients with simple steatosis. This finding is in keeping with the observation that cardiovascular risk is higher in NASH than in simple steatosis. Therefore, NAFLD/NASH should be considered a low-grade chronic inflammatory disease, similar to that observed both in acute and chronic cardiovascular disease.

Oxidative stress plays an important role in the progression from simple steatosis to steatohepatitis [47]. The association between oxidative stress and NAFLD is supported by the detection of lipid peroxidation products and 8-hydroxy-deoxyguanosine in the plasma and liver biopsies from patients with NAFLD [48, 49]. Possible consequences of increased reactive oxygen species (ROS) production include lipid peroxidation, release of proinflammatory cytokines and activation of hepatic stellate cells leading to fibrogenesis [47].

Oxidative stress in an important factor for the pathogenesis and progression of cardiovascular disease and it has been suggested that oxidative stress may be a key link between NAFLD and CVD. Indeed, reactive oxygen species derived from steatosis-stimulated fatty acid oxidation in the mitochondria may further promote the pro-atherogenic oxidative status, which is related to MetS. In fact, the earliest events in the pathogenesis of atherosclerosis are thought to be changes in endothelial functions, in turn triggered by oxidative modification of low-density lipoproteins, leading to the formation of oxidized LDL in the sub-intimal space. Oxidative stress appears to be important in both the early and later stages of the atherosclerotic process. The association of oxidized LDL with the initiation and progression of atherosclerosis could explain the early atherosclerotic process in NASH patients.

NAFLD and metabolic alterations

A strong association between NAFLD and each component of MetS, including central obesity, hypertriglyceridemia and mixed hyperlipidemia, type II diabetes mellitus and hypertension has been clearly demonstrated. IR is considered to play a central role in the pathophysiologic process promoting NAFLD and its progression to NASH. Both peripheral and hepatic IR are implicated in hepatic fat accumulation. Peripheral IR increases triglyceride lipolysis and inhibits esterification of free fatty acids, thus promoting an increased free fatty acids delivery to the liver [50].

According to the two hits hypothesis, IR, a key feature of MetS, is considered to play a central role in the pathogenesis of NAFLD [51, 52]. IR results in hyperinsulinemia and high levels of plasma free fatty acids, which enter into the hepatocyte cytoplasm to produce triglycerides. IR is crucial for NASH development and homeostasis model assessment HOMA–IR has been suggested to predict the early stage of NASH [53]. IR, assessed by HOMA–IR is also predictive of cardiovascular events in patients with type 2 diabetes [54].

NASH is also linked to accelerated atherogenesis through the presence of abnormal production of triglyceride- and cholesterol-rich remnant particles, leading to accelerated atherosclerosis, along with the other features of MetS. Dyslipidemia is characterized by increased serum triglycerides, low high-density lipoprotein (HDL) cholesterol and increased small, dense low-density lipoprotein (LDL) particles, i.e., the so-called ‘atherogenic lipid triad’ [55, 56]. The presence of small dense LDL particles is associated with increased cardiovascular risk. Moreover, it has been observed that patients with NASH have significantly higher postprandial triglyceride levels than healthy control subjects, which represents a further cardiovascular risk factor [57].

Conclusions

NAFLD and NASH have become a major challenge to healthcare systems as the consequence of the increasing rates of obesity worldwide. There is a large body of evidences linking NAFLD to CVD, although only few prospective studies on the relationship of fatty liver with cardiovascular events have been performed. This

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association could be independent or the consequence of MetS and/or an increased cardiovascular disease risk profile.

In fact, NAFLD is strongly associated with the obesity epidemic and its complications, such as IR, chronic inflammation, oxidative stress and atherogenic dyslipidemia and hypertension. NAFLD is also associated with surrogate markers of cardiovascular disease, such as arterial stiffness, endothelial dysfunction, and carotid atherosclerosis.

These findings fit with the hypothesis that NASH contributes to a higher risk of CVD as a component of MetS. However, whether the close association between NAFLD and CVD is 3 explained by the atherogenic profile of MetS or as an independent involvement of NAFLD in the pathogenesis of cardiovascular disease is still under debate. In fact, several studies demonstrated that NAFLD was associated with CVD independently of factors including gender, age, fasting glucose levels, blood pressure, serum lipids, serum creatinine, and, most importantly, waist circumference, i.e., the features of MetS. These findings support the notion that fatty liver is probably not simply another manifestation of MetS, but it may itself induce or worsen IR, type 2 diabetes, and cardiovascular disease. Therefore, the biological mechanisms potentially responsible for accelerated atherogenesis in NAFLD/NASH patients may either have origin in the liver or have the liver as the target of systemic abnormalities.

Recent genetic studies [58, 59] such as those showing a strong correlation between presence of the I148M polymorphism in the PNPLA3 gene and elevated serum levels of ALT and AST and liver inflammation will probably be helpful to improve the assessment cardiovascular risk in NAFLD patients with and without MetS.

Conflict of interest None.

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