TOPIC HIGHLIGHT

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Nonalcoholic fatty liver disease and vascular disease: State-of-the-art

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Abstract

Nonalcoholic fatty liver disease (NAFLD), the most common of chronic liver disease in Western Country, is closely related to insulin resistance and oxidative stress and includes a wide spectrum of liver diseases ranging from steatosis alone, usually a benign and non-progressive condition, to nonalcoholic steatohepatitis (NASH), which may progress to liver fibrosis and cirrhosis. NAFLD is considered the hepatic manifestation of the metabolic syndrome with which shares several characteristics, however recent data suggest that NAFLD is linked to increased cardiovascular risk independently of the broad spectrum of risk factors of metabolic syndrome. Accumulating evidence suggests that the clinical burden of NAFLD is not restricted to liver-related morbidity and mortality, with the majority of deaths in NAFLD patients related to cardiovascular disease and cancer and not to the progression of liver disease. Retrospective and prospective studies provide evidence of a strong association between NAFLD and subclinical manifestation of atherosclerosis (increased intima-media thickness, endothelial dysfunction, arterial stiffness, impaired left ventricular function and coronary calcification). A general agreement emerging from these studies indicates that patients with NASH are at higher risk of cardiovascular diseases than those with simple steatosis, emphasizing the role of chronic inflammation in the pathogenesis of atherosclerosis of these patients. It is very likely that the different mechanisms involved in the pathogenesis of atherosclerosis in patients with NAFLD have a different relevance in the patients according to individual genetic background. In conclusion, in the presence of NAFLD patients should undergo a complete cardiovascular evaluation to prevent future atherosclerotic complications. Specific lifestyle modification and aggressive pharmaceutical modification will not only reduce the progression of liver disease, but also reduce morbidity for cardiovascular disease improving overall prognosis and survival.

Core tip: Nonalcoholic fatty liver disease (NAFLD) is emerging as an independent risk factor for the occurrence and progression of cardiovascular disease. In this review we have systematically analyzed the correlation between NAFLD and cardiovascular diseases (CVD) focusing on the different aspects of CVD (increased carotid intima media thickness, increased arterial stiffness, endothelial dysfunction, impaired left ventricular function, coronary calcification, epicardic fat), on the clinical manifestations (obstructive sleep apnea syndrome, clinical consequences of microangiopathy), and on the underlying physiopathogenic mechanisms (insulin resistance, atherogenic dyslipidemia, inflammation, oxidative stress, adipokynes imbalance, coagulation

imbalance, increased assumption of fructose). Furthermore, we emphasized that NAFLD, by itself, is probably an independent risk factor for the occurrence of CVD.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) represents an emerging public health problem worldwide, has reached epidemic proportions and, currently, is the most common of chronic liver disease in Western Country. NAFLD includes a wide spectrum of liver diseases ranging from steatosis alone, usually a benign and non-progressive condition, to non-alcoholic steatohepatitis (NASH), which may progress to liver fibrosis and cirrhosis. NAFLD is defined by the accumulation of liver fat > 5% per liver weight, in the presence of < 20 g/d for women and < 30 g/d for men of daily alcohol intake; viral or other causes of liver disease must be excluded[1-4]. Patients with NAFLD usually present no manifestation of liver disease and are often identified by elevation of liver enzymes or ultrasonographic evidence of liver steatosis[5].

NAFLD affects about 20%-30% of the general population and is closely related to insulin resistance and oxidative stress[4,6,7]. The reported prevalence of NAFLD varies widely depending on the population studied and definition used and ranges around 20%-25% in the general population, reaching values of 50% and 75% in obese subjects and 65%-90% in those affected by type 2 diabetes[8]. The prevalence was even higher (96%) in obese undergoing bariatric surgery[9]. Interestingly, children are also known to be at risk of developing NAFLD: the prevalence was about 3%-10% in lean subjects and about 53% in obese pediatric population. In the United States, 9-15 millions of people are affected by NASH (prevalence of 3%-5%) of whom 15%-20% may evolve to cirrhosis[10].

In addition to be at risk for NASH, cirrhosis and its complications, NAFLD patients are also at higher risk of systemic and cardiovascular diseases (CVD), including coronary heart disease and stroke[7]. NAFLD is considered the hepatic manifestation of the metabolic syndrome (MS), with which shares several characteristics, and despite the relationship between NAFLD and MS is now increasingly recognized, recent data suggest that NAFLD is linked to increased cardiovascular risk independently of the broad spectrum of risk factors of MS. Indeed, it is hypothesized that NAFLD is not merely a marker of cardiovascular disease but may also be involved in its pathogenesis[7,11-13] (Figure 1).
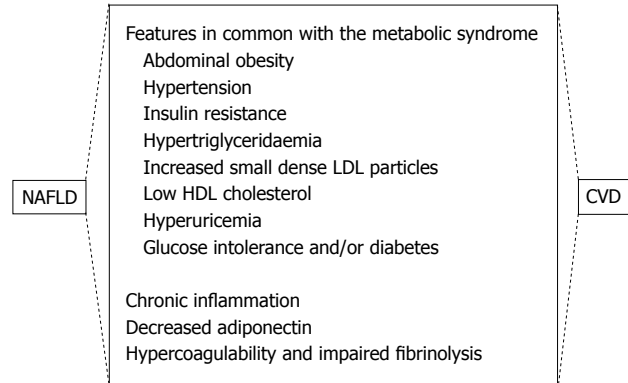
NAFLD, diagnosed either by ultrasonography or liver

Figure 1 Common features in nonalcoholic fatty liver disease and cardiovascular disease. NAFLD: Nonalcoholic fatty liver disease; CVD: Cardiovascular disease.

biopsy, has been associated with a higher prevalence of early asymptomatic cardiovascular alterations such as increased carotid intima-media thickness (IMT) and carotid atherosclerosis[13-15], coronary calcification[13,16], altered left ventricular geometry and early left ventricular diastolic dysfunction[17], and low coronary flow reserve[18]. In a recent study a higher prevalence of NAFLD was also shown in patients with atrial fibrillation[19]. These alterations have been partly associated with the severity of liver damage, defined by both lobular inflammation and fibrosis.

Thus, accumulating evidence suggests that the clinical burden of NAFLD is not restricted to liver-related morbidity and mortality, with the majority of deaths in NAFLD patients related to cardiovascular disease and cancer and not to the progression of liver disease.

In the present review we present an overview on the state of the art of the latest discoveries about the correlation between NAFLD and CVD focusing on the different aspects of CVD (increased carotid intima media thickness, increased arterial stiffness, endothelial dysfunction, impaired left ventricular function, coronary calcification, epicardic fat), on the clinical manifestations [obstructive sleep apnea syndrome (OSAS), clinical consequences of microangiopathy], and on the underlying physiopathogenic mechanism (insulin resistance, atherogenic dyslipidemia, inflammation, oxidative stress, adipokynes imbalance, coagulation imbalance, increased assumption of fructose).

SURVIVAL STUDIES

Recent evidence suggests that NAFLD patients have a decreased survival as compared with general population, and cardiovascular disease dictates the outcome in NAFLD patients more frequently and to a greater extent than does the progression of liver disease[11]. Over 6-28 years of follow-up, several hospital-based studies have reported that patients with NAFLD (biopsy-proven or diagnosed by ultrasound) have a significantly higher all-cause and CVD-related than general population. Adams *et al*[20] in a population-based cohort study, found that 25% of related NAFLD death was linked to acute

myocardial infarction and only 13% to liver disease. In a study published in 2006, which included 212 consecutive patients with biopsy-proven NAFLD, it was found an increased mortality in NASH but not in simple steatosis. Moreover, this study showed that the higher mortality of NASH patients was primarily a result of CVD and, to a lesser extent, of liver-related causes[5]. Similarly, in a study of patients with biopsy-proven NAFLD with 21 years follow-up the main causes of death were CVD and malignancy[21]. Rafiq *et al*[22] in a long-term follow-up evaluation showed that death from coronary artery disease was the most common cause of death followed by malignancy; the liver-related death, including death from hepatocellular carcinoma, was the third most common cause of death. Furthermore, Söderberg *et al*[23] recently confirmed that NASH, but not simple steatosis, was associated with increased mortality from all causes during a 28 years follow-up; cardiovascular disease was the most frequent cause of death followed by extrahepatic cancer and, at third place, by liver-related causes. It should be noted, however, that these studies had a retrospective design and small cohort sizes and the adjustment for potential confounding metabolic factors was not performed[24]. Stepanova *et al*[25] in a combined large cohort of patients with biopsy-proven NAFLD confirmed that cardiovascular (CV) death remains the top cause of death in the NAFLD cohort. However, this retrospective study did not show any difference in CV mortality between NASH and non-NASH subtypes of NAFLD.

A series of prospective studies demonstrated that NAFLD was independently associated with increased risk either of non-fatal CVD events or CVD mortality (Table 1[26]). Targher *et al*[27] using age and sexed matched controls from a diabetes outpatient department, reported that the presence of NAFLD, diagnosed by ultrasound, was independently associated with an increased risk of incident non-fatal CVD events and CVD mortality over 6.5 years of follow-up. Large community and population-based studies showed similar results not only in European but also in Eastern countries[12,28-31]. Lazo *et al*[32] in 2011 and Stepanova *et al*[33] in 2012, using a database of over 11000 US participants found that NAFLD was independently associated with the risk of CVD, but, in contrast with the previous studies, not with increased risk in all-cause mortality. This study, however, was limited by the inclusion of mild hepatic steatosis within the control arm and by using only ultrasonography to exclude hepatic steatosis. Similarly, Kim *et al*[34] in the third NHANES study population found that ultrasonography - defined NAFLD was not associated with overall mortality but, interestingly, NAFLD with advanced fibrosis, was associated with the risk of overall mortality, of which the majority of deaths were due to CVD, even after adjustment for other common risk factors. Also this study had the same limitation of the two above (same database).

Additional long-term prospective and case-control studies are needed to demonstrate the independent risk of premature CVD and decreased survival in patients with well-defined NAFLD/NASH. In particular, understanding how modification of the progression of liver disease could affect and modify CVD progression and outcome is essential.

CAROTID INTIMA MEDIA THICKNESS

Increased IMT of carotid artery, considered a validated parameter of subclinical atherosclerosis and an independent predictor of stroke and myocardial infarction[35-38], and the presence of plaques as index of late atherosclerosis damage, have been used to characterize CVD risk of patients with NAFLD.

Retrospective studies

Several papers reported a strong association between increased IMT and NAFLD. One of the first studies was performed by Targher *et al*[39] in 2004. In this case-control study emerged that in NAFLD patients, all non-obese volunteers, with normal or moderately increased body weight, abdominal visceral fat accumulation was the key mediator for the relationship linking nonalcoholic hepatic steatosis to increased IMT.

Interestingly, many of these studies, conducted among different ethnic populations, showed that NAFLD predicted an increased carotid IMT independently from traditional risk factors, including insulin-resistance[15,40-48]. Fracanzani *et al*[15] in a paired-sample case-control study (125 patients with nonalcoholic fatty liver disease and 250 controls), without a prior diagnosis of diabetes, hypertension, and cardiovascular disease, matched for sex, age, and body mass index found a significant difference in mean values of IMT (0.89 ± 0.26 mm and 0.64 ± 0.14 mm, $P = 0.0001$) and prevalence of plaques [26 (21%) and 15 (6%), $P < 0.001$] between the two groups. In this study independent risk predictors of IMT higher than 0.64 mm (the median value of normal subjects) were presence of steatosis (OR = 6.9), age (OR = 6.0), and systolic blood pressure (OR = 2.3). Aygun *et al*[49] in a population of 40 age-matched-control and biopsy proven NAFLD patients reported that metabolic syndrome and increased IMT were more prevalent in the NAFLD cohort. Kim *et al*[42] in a case-control study stratified by gender concluded that NAFLD was independently associated with carotid atherosclerosis in patients with metabolic syndrome.

Salvi *et al*[50] in the Cardio-GOOSE (Cardio-Gambettola ObservatOry liver Steatosis Estimation) study, a population-based cohort study finalized to evaluate the relationship between NAFLD and subclinical vascular damage, showed that NAFLD was associated with the metabolic syndrome in 48% of cases. IMT values were strongly related to the number of metabolic syndrome factors. In fact no significant differences in IMT were found between controls and patients with NAFLD and no manifestation of metabolic syndrome (0.77 +/- 0.15 mm *vs* 0.76 +/- 0.14 mm). Conversely, in patients with NAFLD and associated metabolic syndrome, IMT values were significantly higher than in patients with NAFLD

Table 1 Prospective and retrospective studies of the risk of cardiovascular mortality in patients with nonalcoholic fatty liver disease diagnosed by liver biopsy and/or by ultrasonography

Ref.	Study design	NAFLD patients	Diagnosis of NAFLD	Mean duration follow-up (yr)	Result
Matteoni *et al*[26], 1999	Retrospective, Hospital-based	132	Histology	18.0	No adjustment made. Higher all-cause, liver-related mortality but no difference in CVD mortality (NASH *vs* simple steatosis)
Dam-Larsen *et al*[21], 2004	Retrospective, Hospital-based	109	Histology	16.7	Analysis performed by gender. No difference in all-cause and liver-related or CVD mortality (simple steatosis *vs* general population)
Adams *et al*[20], 2005	Retrospective community-based	420	US and/or histology	7.6	Matched for gender, age and country Higher all-causae, liver-related and CVD mortality (CVD is the second cause of death by frequency) in NAFLD patients (especially cirrhosis)
Targher *et al*[12], 2005	Prospective case-control, Hospital-based	248/2103[1]	US	5.0	NAFLD was independently associated with increased nonfatal CVD and CVD mortality
Ekstedt *et al*[5], 2006	Retrospective, Hospital-based	129	Histology	13.7	Matched for gender, age and country Higher all-cause, liver-related and CVD mortality (NASH but no simple steatosis *vs* general population)
Hamaguchi *et al*[28], 2007	Prospective, community-based	312/1637[2]	US	5.0	NAFLD was independently associated with increased risk of nonfatal CVD events
Targher *et al*[27], 2007	Prospective, Hospital-based	1421/2103[1]	US	6.5	NAFLD was independently associated with increased risk of nonfatal CVD events and CVD mortality
Haring *et al*[29], 2009	Prospective, community-based	2490/4160[3]	US and altered GGT	7.3	NAFLD was independently associated with increased risk of all-cause and CVD mortality in men
Rafiq *et al*[22], 2009	Retrospective, Hospital-based	173	Histology	13.0	No adjustments made Higher liver-related mortality, but no difference in overall mortality (NASH *vs* simple steatosis)
Söderberg *et al*[23], 2010	Retrospective, Hospital-based	118	Histology	18.0	Matched for gender, age and year Higher all-cause, liver-related and CVD mortality (NASH but no simple steatosis *vs* general population)
Lazo *et al*[32], 2011	Prospective, population-based (third NHANES study population 1988-1994)	2089/11371[4]	US	14.3	Independent increased risk of CVD but no increased risk in all-cause, liver-related and CVD mortality
Stepanova *et al*[33], 2012	Prospective, population-based (third NHANES study population 1988-1994)	2492/11371[4]	US	14.3	Independent increased risk of CVD but no increased risk in all-cause, liver-related and CVD mortality
Treeprasertsuk *et al*[31], 2012	Prospective, community-based	309	US, CT, MRI, and or Histology	11.5	Higher 10-Year CVD risk (NAFLD *vs* general population)
Kim *et al*[34], 2013	Prospective, population-based (third NHANES study population 1988-1994)	4083/11154[4]	US and NAFLD Fibrosis Score	14.5	US-Defined NAFLD was not associated with overall mortality. NAFLD with advanced fibrosis was independently associated with overall mortality (majority of deaths were due to CVD)
Stepanova *et al*[25], 2013	Retrospective, Population-based	289	Histology	6.25	No adjustments made Higher risk of liver-related mortality in NASH than non-NASH. NAFLD and type II diabetes highest risk for overall and liver-related mortality

[1]Patients with type 2 diabetes; [2]Participants of health checkup program; [3]West Pomerania population; [4]United Stated adult population. US: Ultrasonography; CT: Computed tomography; MRI: Magnetic resonance imaging; CVD: Cardiovascular disease; NAFLD: Nonalcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis.

alone (0.85 +/- 0.16 mm, $P < 0.005$)[50]. From a large population based survey of over 8500 middle aged and elderly Chinese population, Huang *et al*[46] reported that NAFLD was associated with elevated IMT independently of the conventional risk factors. Thakur *et al*[51] in a case-control study in non-diabetic population reported that NAFLD seemed to be an independent predictor of having high average IMT, independently of obesity and metabolic syndrome. Notably, this is the first study in Asian Indians demonstrating the association.

The association between NAFLD and increased carotid IMT was independent of the metabolic syndrome in at least four studies[44-47].

Increased IMT and liver damage

It has been reported that subclinical cardiovascular disease was significantly associated with the severity of liver disease. Targher *et al*[52] compared carotid IMT in 85 consecutive patients with biopsy-proven NAFLD and 160 age-, sex-, and BMI-matched healthy control

subjects and found that the severity of liver histopathology in NAFLD patients was strongly associated with early carotid atherosclerosis, independently of classical risk factors, insulin resistance, and the presence of metabolic syndrome, suggesting that the severity of liver damage could play a role in atherosclerosis development. Băloşeanu *et al*[53] reported that the severity of liver histopathological lesions among NAFLD patients was strongly associated with increased IMT. This study, however, had a limitation because it was performed in a small sample of NAFLD subjects. Also Kucukazman *et al*[54] in a recent study found that the mean carotid IMT was significantly increased in patients with NAFLD and showed a significant positive cancel weak correlation between ultrasonographic steatosis grade and mean IMT ($r = 0.376$, $P < 0.001$).

IMT and type 2 diabetes
At least four studies examined the link between NAFLD, type 2 diabetes and subclinical atherosclerosis damage. It is well known that NAFLD is extremely common in people with type 2 diabetes and is mainly associated with uncontrolled diabetes. Targher *et al*[55] studied a population of 200 diet-controlled type 2 diabetic subjects, reported that NAFLD patients had a markedly greater carotid IMT and that the increase of carotid IMT was largely explained by HOMA-estimated insulin resistance. In contrast IMT values were found to be significantly higher in diabetic patients regardless of the degree of steatosis by Cakir *et al*[56]. Similarly other two studies performed in diabetic population reported that hepatic steatosis was not associated with carotid atherosclerosis and suggested that the association of hepatic steatosis and cardiovascular disease might be just an epiphenomenon[57,58].

Pediatric population
One of the first evidence of the association between fatty liver and atherosclerosis were the autoptic findings in 817 children who died of external causes showing that fatty liver was present in 15% of the children, mild atherosclerosis in 21% and moderate to severe atherosclerosis in 2%. Atherosclerosis was found more common in children with fatty liver (30%) than those without fatty liver (19%). Interestingly, growing evidence suggests a strong relationship between early carotid atherosclerosis and NAFLD, although longitudinal studies are needed to clarify the extent to which pediatric NAFLD and its severity influence long-term cardiovascular outcomes in the general population[59-65].

In conclusion, considering the large number of publications on the relationship between IMT and vascular damage, carotid screening for NAFLD might be beneficial for assessment of future atherosclerotic complications.

ARTERIAL STIFFNESS
Measurement of biomechanical properties of arteries has become an important surrogate outcome used in ep-idemiological and interventional cardiovascular research. A loss of arterial elastic properties results in a range of linked pathophysiological changes in the circulation including increased pulse pressure, left ventricular hypertrophy, subendocardial ischaemia, vessel endothelial dysfunction and cardiac fibrosis[66]. Arterial stiffness, determined by measurement of pulse wave velocity, has been shown to be an independent indicator of symptomatic cardiovascular disease[67]. Carotid and femoral pulse wave velocity has been considered the noninvasive gold standard measure of arterial stiffness[50]. Brachial-ankle pulse wave velocity is also widely used to screen for atherosclerotic vascular damage[13,67]. Several recent studies reported a strong association between NAFLD and impaired arterial elastic properties. In the GOOSE study Salvi *et al*[50] showed that NAFLD may play an independent role in determining arterial stiffness. Vlachopoulos *et al*[67] in a case-control study with biopsy-proven NAFLD reached similar results. Huang *et al*[46] in the study including 8632 Chinese men aged $\geqslant$ 40 years showed that brachial-ankle pulse wave velocity was increased in NAFLD independently of conventional cardiovascular disease risk factors and metabolic syndrome. In another study of the same author performed in adolescents it was found that NAFLD was only associated with increased arterial stiffness in the presence of the "high risk" metabolic cluster, suggesting that arterial stiffness related to the presence of NAFLD was predicted in adolescents by the presence of an adverse metabolic profile[68]. Kim *et al*[69] in a case-control study indicated that the presence and the degree of NAFLD were associated with arterial stiffness, measured by brachial-ankle pulse wave velocity, in non-hypertensive, non-diabetic individuals, especially in those without metabolic syndrome. Similarly, Lee *et al*[70] showed that arterial stiffness, was independently associated with the risk for NAFLD regardless of classical CVD risk factors.

Işilak *et al*[71] and Fotbolcu *et al*[72] reported that elastic properties of aorta were abnormally changed in patients with NAFLD, probably related to or associated with insulin resistance[71,72]. In contrast, Catena *et al*[73] revealed that in essential hypertensive patients without additional cardiovascular risk factors, NAFLD was associated with insulin resistance but not with increased arterial stiffness.

ENDOTHELIAL DYSFUNCTION
Endothelial dysfunction is one of the earliest markers of atherosclerosis. Recent studies showed an association between NAFLD and endothelial dysfunction, assessed by brachial artery flow mediated dilatation (FMD). In 2005 Villanova *et al*[74] reported that FMD was significantly reduced in NAFLD population; in this case-control study NAFLD, diagnosed by liver biopsy or by ultrasound, predicted a reduced FMD after adjusting for age, sex, BMI and insulin resistance. Senturk *et al*[75] also reported that endothelial dysfunction was worse in NASH compared to simple steatosis and controls suggesting that inflam-

mation in the liver plays a role.

Mohammadi *et al*[44] in a study performed in 2011, reported that pure NAFLD without metabolic syndrome in middle-aged subjects was strongly associated with morphological (increased carotid IMT) and physiological flow-mediated changes.

In a cohort of never treated uncomplicated hypertensive outpatient subjects the presence of NAFLD was associated with physiological FMD changes and insulin-resistance[76]. Thakur *et al*[51] in a case-control study reported that in a cohort of Asian Indians subjects NAFLD is significantly associated with subclinical atherosclerosis and endothelial dysfunction. Interestingly, these results were independent from the classical cardiovascular risk factors, *i.e.*, obesity and metabolic syndrome. In another observational case-control study the measurement of brachial artery FDM was significantly lower in patients with NAFLD group compared to control group suggesting that NAFLD, and in particular NASH, is associated with endothelial dysfunction, independently from metabolic syndrome[48]. Similar results were reported by Kucukazman *et al*[54]. In addition plasma levels of fetuin-A, which is known to inhibit insulin signalling and recently emerged as a biomarker for diabetes risk, was found independently associated with endothelial dysfunction and subclinical atherosclerosis in NAFLD patients[77].

IMPAIRED LEFT VENTRICULAR FUNCTION

At least seven studies reported that NAFLD was associated with impaired left ventricular diastolic dysfunction, alteration in energy metabolism and disturbance of cardiac rhythm (Table 2). Goland *et al*[78] found that patients with NAFLD, in the absence of morbid obesity, hypertension, and diabetes had early features of left ventricular diastolic dysfunction and mildly altered left ventricular (LV) geometry. Lautamäki *et al*[79] found that in patients with type 2 diabetes and coronary artery disease, liver fat content was a novel independent indicator of myocardial insulin resistance and reduced coronary functional capacity. Fallo *et al*[80] in a study conducted in a cohort of never-treated essential hypertensive patients with nonalcoholic fatty liver disease, reported that NAFLD was significantly associated with insulin resistance and abnormalities of left ventricular diastolic function, suggesting a concomitant increase of metabolic and cardiac risk. On the contrary, Perseghin *et al*[81] reported that in newly diagnosed individuals with fatty liver no impaired LV morphological features and systolic and diastolic functions were present but they had abnormal LV energy metabolism that correlated with higher mediastinal visceral fat. In a recent study in which 50 consecutive patients with type 2 diabetes were enrolled it was observed that in patients with NAFLD LV morphology and systolic function were preserved but early features of LV diastolic dysfunction were present[17].

Hallsworth *et al*[82] in a cohort of nineteen adults with

NAFLD age-, sex-, and BMI-matched to healthy controls without liver or metabolic diseases, reported that adults with NAFLD had significantly thicker left ventricular wall at systole (14 ± 3 mm *vs* 12 ± 2 mm, $P < 0.01$) and diastole (8 ± 1 mm *vs* 7 ± 1 mm, $P < 0.01$) than those without fatty liver and showed decreased longitudinal shortening (14% ± 3% *vs* 17% ± 3%, $P < 0.01$). This case-control study concluded that significant changes in cardiac structure and function are evident in adults with NAFLD in the apparent absence of metabolic changes or overt cardiac disease.

Interestingly, a recent study demonstrated that NAFLD was associated with an increased risk of incident atrial fibrillation (AF), (OR = 4.49, 95%CI: 1.6-12.9, $P < 0.005$). Adjustments for age, sex, hypertension and electrocardiographic features (left ventricular hypertrophy and PR interval) did not attenuate the association between NAFLD and incident AF (adjusted OR = 6.38, 95%CI: 1.7-24.2, $P = 0.005$)[19].

CORONARY CALCIFICATION

Computed tomography examination of the coronary arteries can detect calcification indicative of arterial disease in asymptomatic people which has been identified as a surrogate marker of subclinical atherosclerosis[83]. Coronary artery calcification (CAC) was considered an independent marker of CVD risk[84]. Akabame *et al*[85] in a case-control study, evaluating vulnerable coronary plaques and steatosis, by multi-slice computed tomography, showed that liver fat was significantly correlated with lipid core plaques. Adjusted odds ratio of NAFLD for remodeling lesions was 2.41 (95%CI: 1.24-4.67; $P = 0.009$), and those for lipid core lesions 2.29 (95%CI: 1.15-4.56; $P = 0.018$)[85]. A population study performed in 2007, evaluating 371 non-diabetic, asymptomatic middle-aged men free of known coronary heart disease, showed that the presence of hepatic steatosis was associated with a greater prevalence of risk factors for atherosclerosis, with a higher 10-year total coronary heart disease risk (calculating by Framingham Risk Score) and was independently associated with CAC in subjects with metabolic syndrome[86].

Three studies published in 2010 reported similar results. Assy *et al*[87] found that patients with NAFLD, even without metabolic syndrome, are at high risk for atherosclerosis and had a higher prevalence of coronary calcification. In a retrospective study, Chen *et al*[88] reported that besides traditional risk factors, such as male gender, increased age, and DM, NAFLD was also associated with moderate to high risk of coronary disease and with the severity of CAC. Jung *et al*[89] found, in a cohort of 1218 Korean subjects, that NAFLD predicted coronary calcification. Individuals with combined elevated ALT (> 30 U/L) and ultrasound diagnosed hepatic steatosis had higher odds of a CAC score > 100 compared to controls and individuals with elevated ALT or steatosis alone. However it is not possible to understand from the paper whether the patients with increased ALT had NASH.

Table 2 Studies of the impaired left ventricular function and disturbance of rhythm in patients with nonalcoholic fatty liver disease

Ref.	Study design	Study population	Study size	Diagnosis of NAFLD	Cardiac parameters examined	Results
Left ventricular function						
Goland et al[78], 2006	Retrospective, Case-control study, Hospital-based	NAFLD vs control	38 NAFLD 25 non-NAFLD	US or histology	Complete echocardiographic study, TDI, peak velocities of E and A diastolic filling, E/A ratio, V_p, E', and S' of mitral annulus	NAFLD patients had increased thickening of the intraventricular septum and posterior, lower E diastolic filling velocity, lower E/A ratio, longer, lower V_p and lower E'. No differences were found according to LV systolic function
Lautamaki et al[79] 2006	Retrospective, Case-control study, Hospital-based	Patients with type 2 diabetes and CVD	28 low liver fat 27 high liver fat. Matched for age, BMI, fasting glucose	H-MRS	Positron emission tomography. Myocardial perfusion was measured with[15O]H2O and myocardial and skeletal muscle glucose uptake with 2-deoxy-2-[18F]fluoro-D-glucose during hyperinsulinemic euglycemia	Liver fat content is independently associated with impaired myocardial metabolism
Fallo et al[80] 2009	Retrospective, Case-control study, Hospital-based	Never-treated essential hypertensive patients	48 NAFLD 38 non-NAFLD. Matched for sex, age and blood pressure levels	US	Complete echocardiographic study: TDI, peak velocities of E and A diastolic filling, E/A ratio, V_p, E', and S' of mitral annulus	NAFLD patients had increased prevalence of left ventricular hypertrophy, diastolic dysfunction that increased according to the degree of NAFLD
Perseghin et al[81] 2008	Retrospective, Case-control study, Hospital-based	Young non-diabetic men	21 fatty liver 21 non fatty liver. Matched for age, BMI, blood pressure levels, lipid values	H-MRS	MRI and MRS	No difference in morphological parameters of the LV, systolic and diastolic functions. NAFLD patients had reduced values of PCr/ATP ratio
Bonapace et al[17] 2012	Retrospective, Case-control study, Hospital-based	Patients with type 2 diabetes	32 NAFLD 18 non-NAFLD. Matched for age, sex, BMI, waist circumference, hypertension, smoking, diabetes duration, microvascular complication status, medication use	US	Complete echocardiographic study, TDI, peak velocities of E and A diastolic filling, E/A ratio, V_p, E', and S' of mitral annulus	NAFLD patients had lower E', tissue velocity, higher E-to-e' ratio, higher time constant of isovolumic relaxation, higher LV-end diastolic pressure (EDP), higher LV EDP/end diastolic volume. No difference in morphological parameters of the LV, systolic functions
Hallsworth et al[82] 2013	Retrospective, Case-control study, Hospital-based	NAFLD and healthy controls	19 NAFLD 19 non-NAFLD. Matched for age, sex, BMI, weight, and body surface area vs control	H-MRS	MRI and MRS	NAFLD patients had thicker left ventricular walls at systole and diastole, decreased longitudinal shortening, higher concentric remodelling. No difference in PCr/ATP ratio
Disturbance of cardiac rhythm						
Targher et al[19] 2013	Prospective, Hospital-based	Type 2 diabetes	281 NAFLD 119 non-NAFLD. Adjustments for age, sex, hypertension and electrocardiographic features	US	12-lead electrocardiogram	NAFLD was associated with an increased risk of incident AF

A: Late; BMI: Body mass index; E: Early; E': Early diastolic velocity; H-MRS: Proton magnetic resonance spectroscopy; cardiac MRI: Magnetic resonance imaging; MRS: 31P-MR spectroscopy; NAFLD: Nonalcoholic fatty liver disease; PCr/ATP ratio: Phosphocreatine/adenosine triphosphate ratio; S': Systolic velocity; TDI: Tissue Doppler imaging; US: Ultrasonography; Vp: Flow propagation velocity; AF: Atrial fibrillation.

Kim et al[16] in the largest population-based study published in 2012 showed a strong relationship between NAFLD and CAC; 4123 cases and controls from Seoul were enrolled. The study reported that NAFLD predicted a CAC score > 10; computed tomography (CT) examination revealed that visceral adiposity attenuated but did not eliminate the

relationship between NAFLD and CAC (adjusted OR = 1.32 (95%CI: 1.12-1.57)[16]. These recent evidences suggested that detection of NAFLD could signal the existence of an increased coronary artery disease risk independent of visceral adiposity.

Only two studies showed no significant association between NAFLD and coronary calcification, probably one for the different selection of patients[57] and the other for the small series of patients (70 subjects)[90].

OBSTRUCTIVE SLEEP APNEA SYNDROME

OSAS is a common medical condition that affects about 4% of general population with higher prevalence among obese subjects (incidence 25%-35%)[91]. Besides obesity, age, male, sex, smoke and alcohol intake are risk factors for OSA. OSAS is a form of sleep disordered breathing, which is characterized by repetitive complete and/or partial collapses (apnea and hypopnea respectively) of the upper airways. OSAS is characterized by episodes of chronic intermittent hypoxia and sleep fragmentation, with increase in sympathetic activity and promotion of oxidative stress, pro-inflammatory cytokine production, platelet aggregation, endothelial dysfunction and metabolic dysregulation. All these mechanisms provide the pathophysiological basis for the increased risk of CVD observed in these patients[92].

OSAS has been identified as an independent comorbid factor in cardiovascular and cerebrovascular diseases, and physiopathological links may exist with onset and progression of heart failure. It has also been found an association between OSAS and the manifestations of the metabolic syndrome, in particular with obesity[91], and with arterial stiffness, endothelial dysfunction and carotid IMT[93]. The disease is classified as mild, moderate and severe based on the number of apneas and/or hypopneas per hour of sleep, known as the apnea-hypopnea index. This is assessed by polysomnography (PSG) or other forms of sleep monitoring[94]. Both OSAS and metabolic syndrome, of which NAFLD represents the hepatic manifestation, may exert negative synergistic effects on the cardiovascular system through multiple mechanisms (*e.g.*, hypoxemia, sleep disruption, activation of the sympathetic nervous system, and inflammatory activation). It has been found that continuous positive airway pressure therapy for OSAS provides an objective improvement in symptoms and cardiac function, decreases cardiovascular risk, improves insulin sensitivity and adiponectin level, reduces adipocytokines (leptin and resistin) levels, and normalized biomarkers[95].

Recent experimental evidence connected chronic intermittent hypoxia with insulin-resistance and reported that intermittent hypoxia concurred to the pathogenesis of NAFLD. Moreover, in NAFLD patients, the presence of OSAS resulted linked to the progression of NAFLD to NASH[91].

NAFLD AND MICROANGIOPATHY

It has been reported that NAFLD may also be associated with micro-vascular changes in other organs that have direct or indirect influence on CVD and may accelerate the presentation of CVD. For example, patients with NAFLD, in a type 2 diabetic population, had significant higher age- and sex-adjusted prevalence rates of both non-proliferative (39% *vs* 34%) and proliferative/laser treated retinopathy (11% *vs* 5%) and of chronic kidney disease (15% *vs* 9%) than counterparts without NAFLD. Furthermore, in this study, NAFLD was associated with an increased incidence of chronic kidney disease, independent of classical risk factors[96]. At least three studies showed an association between retinal vascular changes and NAFLD. Băloşeanu *et al*[53] in 10 biopsy-proven NAFLD patients, found a connection between the degree of NAFLD and the retinal vascular changes in those with carotid atherosclerosis. Josef *et al*[97] analyzing a cohort of non-diabetic and non-hypertensive patients, found that fatty liver (OR = 2.5; *P* < 0.01), IMT (OR = 2.3; *P* < 0.001), and retinal changes (OR = 1.5; *P* < 0.01) were strongly associated with CAD independent of metabolic syndrome (OR = 1.2; *P* < 0.05). Also Targher *et al*[98] suggested that patients with NAFLD have an increased prevalence of chronic kidney disease, known risk factor for CVD. An increasing number of studies showed that NAFLD may be an independent risk factor for chronic kidney disease[96,98-108]. In several retrospective studies the prevalence of chronic kidney disease defined as eGFR < 60 mL/min/1.73 m^2 or proteinuria- and/or microalbuminuria were significantly higher among NAFLD patients compared with those without[100,101,103]. Interestingly, the majority of these studies reported that NAFLD, and in particular NASH, was independently associated with chronic kidney disease /microalbuminuria even after adjusting for traditional risk factors, *i.e.*, age, sex, BMI, hypertension, diabetes, smoking and hyperlipidemia[100,102,104,109]. However some studies defined NAFLD according to elevated of GGT or ALT, and thus they need to be interpreted with caution due to the relative lack of diagnostic accuracy. In addition Neri *et al*[110] reported that, independently related with the traditional risk factors for cardiovascular disease, nonspecific inflammation and oxido-reductive imbalance may play an important role in the progression of NAFLD and atherosclerotic disease in hemodialysis patients.

In conclusion NAFLD, in particular biopsy proven NASH, was associated with a greater prevalence of microvessel damage, in particular chronic kidney disease.

EPICARDIC FAT

Ectopic fat accumulation within and around organs such as the heart and the liver are linked to an increased cardiovascular risk[111,112]. The epicardial fat, is considered the true visceral fat and the best representation of fatty heart[113]. Epicardial fat displays peculiar biomolecular

and anatomic characteristics, and its direct contiguity to myocardium reflects the intra-myocardial fat accumulation. Epicardial adipose tissue might secrete vasoactive products that regulate coronary arterial tone, in addition various studies have highlighted the potential importance of adipose tissue in relation to inflammatory burden in cardiovascular diseases. In fact epicardial fat has been demonstrated to be a source of several pro-inflammatory and proatherogenic cytokines that could influence the cardiac function[114-116]. It has been reported that epicardial fat also correlates with several cardiac comorbidities, including coronary artery disease, left ventricular dysfunction, atrial fibrillation; moreover a pivotal role of body fat has been suggested in the development and progression of both diastolic and systolic heart failure[117,118].

Epicardial fat has been found clinically related to abdominal visceral adiposity, coronary artery disease, subclinical atherosclerosis and metabolic syndrome[119-121]. In particular Lai *et al*[122] reported graded increases in fasting glucose, insulin resistance, and alanine transaminase levels across higher tertiles of epicardial fat thickness. In addition the measurement, by magnetic resonance imaging, of epicardial fat, as well as the degree of hepatic steatosis, correlates with abdominal adiposity and hypertriglyceridemia. A good relation between epicardial fat thickness and the degree of fatty liver severity was described in adult and pediatric patients with NAFLD and obesity[65,123], and with early atherosclerotic damage[45].

The imaging tools used to study and quantify epicardial fat were cardiac CT and magnetic resonance imaging (MRI). Recently, Lai *et al*[122] showed that ultrasonographic measurement of EAT thickness highly correlated with that measured with CT.

GENETIC

In the last few years, growing evidence indicates that in all diseases, including cardiovascular diseases, environment and genetic factors cooperate in inducing disease manifestation. Data arising from genome-wide (GWAS) or candidate association studies identified single nucleotide polymorphisms (SNPs) in genes involved in metabolic homeostasis, inflammation, oxidative stress and fibrogenesis, as associated with NAFLD and its severity. Patatin-like phospholipase-3 (PNPLA3)/adiponutrin, rs738409 C.G SNP, remains the most validated risk gene in this setting[124]. A recent study by Valenti *et al*[125] showed that PNPLA3 variant was directly related to hepatic apoptosis in NAFLD. According to these data, it is plausible to hypothesize that PNPLA3 genotype, by regulating apoptotic activity, may be also involved in the pathogenesis of atherosclerosis and might modulate vascular damage. Recently, Petta *et al*[126] showed that PNPLA3 GG genotype was associated with higher severity of carotid atherosclerosis in younger patients with NAFLD. PNPLA3 gene variants might increase lipid storage in the arterial vessels and could also induce release of ICAM-1, an endothelium-derived inflammatory

marker that has been associated with myocardial infarction and stroke[127].

It was also hypothesized that the same pathways generating liver disease might also be involved in vascular damage.

RELATION BETWEEN NAFLD AND ATHEROSCLEROSIS INDEPENDENTLY OF KNOWN RISK FACTORS: POSSIBLE MECHANISMS

Growing evidence suggests that patients with NAFLD are at higher risk of cardiovascular disease than those without NAFLD, even when their primary disease (diabetes, hypertension…) has a strong potential for atherosclerosis[9,11,13].

Several mechanisms can be involved in atherosclerosis acceleration in patients with NAFLD, including genetic predisposition, insulin resistance and atherogenic dyslipidemia, oxidative stress, chronic inflammation, reduced levels of the adiponectin (an adipocytochine with antiatherogenic and antidiabetic properties), and altered production of pro and anticoagulant factors. All these mechanisms are present at the same time (Figure 2)[11,128].

The association between early atherosclerosis and NAFLD has been extensively described and summarized in Table 3 in which are reported variables independently associated with IMT in adult subjects[7,13,15,25,41-44, 46,47,49-52,55,58, 110,125,129-133].

INSULIN RESISTANCE

Normally insulin inhibits adipose tissue lipolysis and hepatic VLDL secretion by the liver, and on the contrary, insulin resistance promotes fatty acid accumulation in the liver which in turn causes an increase in insulin resistance responsible for a reduced suppression of endogenous liver glucose production. Given the very close relationship between NAFLD and insulin resistance[134,135], which is present in more than 90% of patients with NAFLD, it is still debated which one of the two is the earlier event. In the presence of overweight/obesity and of increased circulating insulin, due to insulin resistance, there is increased lipolysis of adipose tissue which leads to increased flux of free fatty acid (FFA) to the liver from all lipid depots, including ectopic fat. In addition hepatic lipid accumulation occurs also because of dietary chilomicrons and *de novo* intrahepatic lipogenesis. Further accumulation of lipids in the liver is, in turn, responsible for insulin resistance worsening with a consequent vicious circle. In addition, besides the augment of FFA, the increased release of different molecules from visceral adipose tissue, including inflammatory cytokines, leads to adipose tissue inflammation which aggravates insulin resistance and facilitates atherosclerosis development and cardiovascular disease. Experimental evidences indicate that activation of proinflammatory pathways occurs mainly through the nuclear
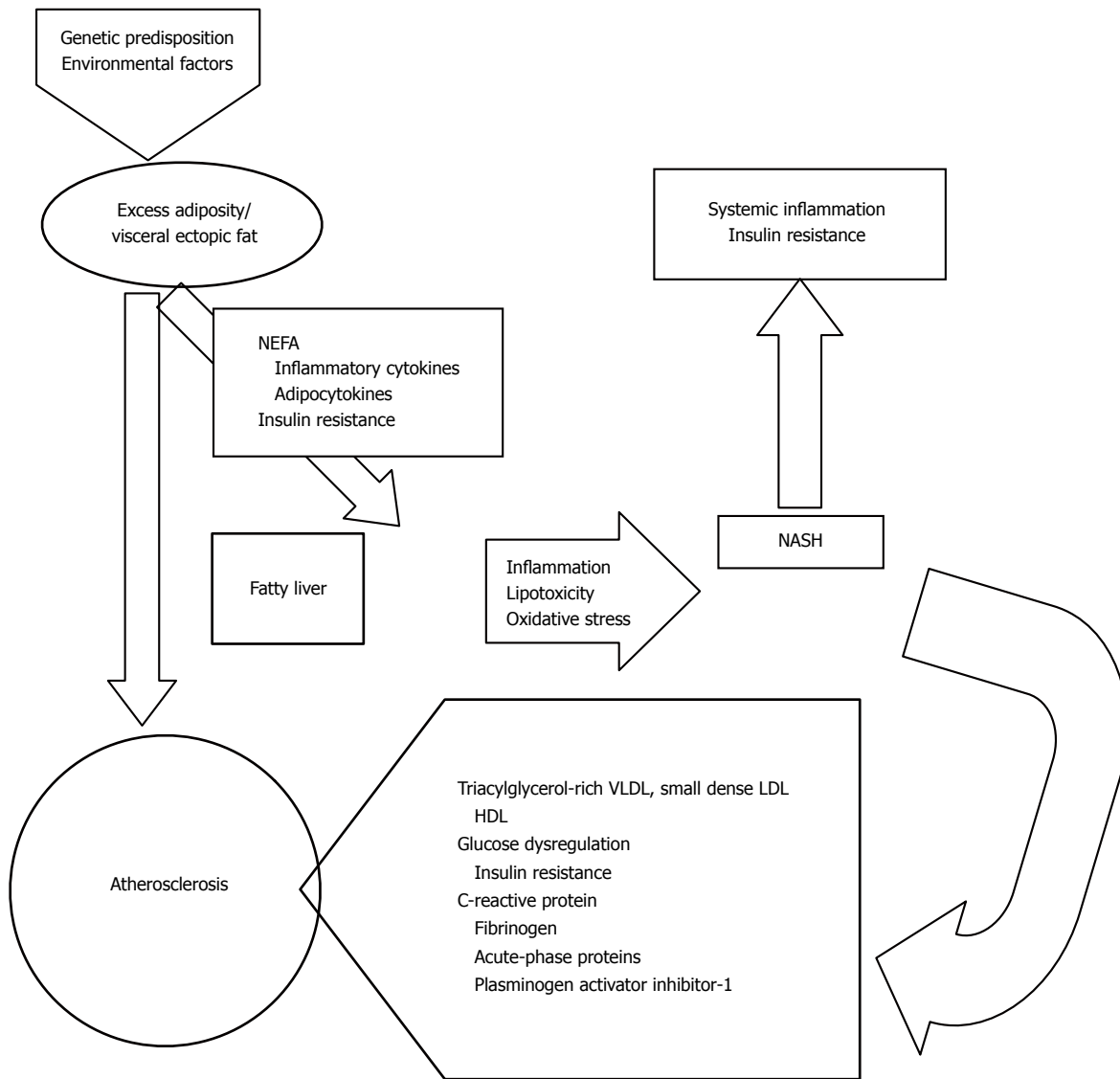
Figure 2 Mechanisms potentially responsible for atherosclerosis in nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. NASH: Non-alcoholic steatohepatitis.

factor κB (NF-κB) and the c-Jun N-terminal kinase (JNK) pathways which cooperate in inducing insulin resistance which, in association with several factors, including chronic inflammation, atherogenic dyslipidemia, hypercoagulation and hypofibrinolysis, and liver involvement either as target of the metabolic abnormalities or as producer of proatherogenic molecules, triggers arterial damage leading to accelerated atherosclerosis. It is possible that in lean subjects, which are roughly 10%-15% of patients with NAFLD, the insulin resistance and chronic inflammation of visceral adiposity is at least initially replaced by insulin resistance in skeletal muscle[11].

ATHEROGENIC DYSLIPIDEMIA

Typical atherogenic dyslipidemia is frequently detected in NAFLD, characterized by high triglycerides and low HDL-C which, as above reported, together with insulin resistance and visceral adiposity play a major role in ac-

celerated atherosclerosis[136-138]. A high level of low density lipoprotein (LDL) cholesterol, in particular of small dense LDL which are more atherogenic than type A LDL particles, is frequently detected in patients with NAFLD as well as increased levels of oxidized LDL, which are highly atherogenic. Moreover, in a recent large, multiethnic, gender balanced cohort, NAFLD was associated with the atherogenic dyslipidemia phenotype (higher fasting serum triglycerides and lower serum HDL) in a dose dependent fashion, suggesting a possible independent pathophysiologic role between NAFLD and dyslipidemia[139]. The main alteration believed to play a key role in atherosclerosis is the overproduction of larger TG-rich VLDL particles from the liver. Furthermore, as described in the insulin resistance paragraph, in the presence of increased adipose tissue there is an increased flux of FFA from adipose tissue to the liver where are re-esterified into triglycerides with consequent increased VLDL secretion into the blood stream. In addition, increased lipid

Table 3 Studies on the association between non-alcoholic fatty liver disease and carotid intima-media thickness in adults: evaluation of the independent predictors for intima-media thickness increase

Ref.	cases *n*	Cohort	NAFLD diagnosis by	Independent predictors for IMT
Brea *et al*[40] 2005	80	NALFD and matched population controls	US	NAFLD, age, serum ferritin
Volzke *et al*[41] 2005	1261 + 2961	German population	US	Hepatic steatosis
Lonardo *et al*[129] 2006	449	Population/NAFLD	US	Age
Targher *et al*[52] 2006	145	NAFLD and matched healthy controls	Biopsy	Severity of histological feature of NAFLD
Targher *et al*[55] 2006	200	Type2 diabetes +/- NAFLD	US	HOMA-IR
Aygun *et al*[49] 2008	80	NAFLD and matched healthy controls	US	NAFLD and BMI
Sookoian *et al*[7] 2008 (Meta-regression analysis)	3497	Subjects	US	ALT, GGT, NAFLD
Fracanzani *et al*[15] 2008	375	NAFLD and matched population controls	US and biopsy	Steatosis, age and blood pressure
Kim *et al*[42] 2009	1021	Cross sectional	US	NAFLD with MS
Petit *et al*[58] 2009	101	Type 2 diabetes	MRS	Age, no association with steatosis
Ramilli *et al*[43] 2009	154	Referred subjects for abdominal US	US	NAFLD
Wang *et al*[130] 2009	170	Healthy subjects	US	ALT levels
Salvi *et al*[50] 2010	220	NAFLD, healthy controls		NAFLD and MS
Mohammadi *et al*[44] 2011	335	Male NAFLD controls	US	NAFLD with or without MS
Neri *et al*[110] 2011	90	Chronic hemodialysis plus steatosis and healthy controls	US, biopsy	Histological steatosis
Valenti *et al*[131] 2011	506	NAFLD	US	Systolic blood pressure, glucose, LDL, abdominal circumference, age, ferritin
Huang *et al*[46] 2012	2590 + 6042	Chinese population +/- NAFLD	US	NAFLD
Kang *et al*[47] 2012	633	Non-diabetic +/- NAFLD	US	NAFLD with or without MS
Thakur *et al*[51] 2012	80	Non-diabetic NAFLD, healthy controls	US	NAFLD
Kim *et al*[132] 2013	769	Healthy subjects	US	Upper normal range of ALT in women with NAFLD
Oni *et al*[13] 2013 Systematic review	16 studies	Population, cross- sectional, case-control	US, biopsy	Positive association between IMT and NAFLD
Petta *et al*[126] 2013	162 + 267	NAFLD and validation cohort	Biopsy	PNPLA3 polymorphism in younger patients
Kim *et al*[133] 2014	4437	Type 2 diabetes	US	NAFDL with insulin resistance

US: Ultrasonography; MS: Metabolic syndrome; IMT: Intima-media thickness; NAFLD: Nonalcoholic fatty liver disease; MRS: Magnetic resonance spectroscopy.

availability within the liver and insulin resistance inhibit apolipoprotein B (APO-B) degradation with formation of more VLDL. The increased level of APO-B, a large protein involved in the transport of triglycerides and cholesterol from the liver to peripheral tissues, which is the primary protein component of the atherogenic LDL particles, is also responsible for accelerated atherosclerosis. The altered ratio between APO-B and apolipoprotein A-1 (APO-A1), the protein component of the anti-atherogenic HDL particles, has been suggested to be a good predictor of cardiovascular risk, even better than HDL/LDL ratio, with more cholesterol deposited in the arterial wall when the ratio is higher[140-142].

INFLAMMATION

Several results obtained in animal models have shown that lipid accumulation in the liver leads to sub-acute hepatic inflammation followed by cytokine production *via* the NF-κB pathway, in turn followed by increase in insulin resistance. Activation of the NF-kB pathway in the liver of patients with NASH leads to increased transcription of several pro-inflammatory gene that mediate the progression of systemic, low-grade inflammation. In line with these results a growing body of evidence indicates the atherosclerosis is proportional to the severity

of liver damage, with CVD accelerated in patients with NASH but to a much less extent in those with simple steatosis[143]. Although the relation between hepatic inflammation and atherosclerosis is still matter of debate, these results are very suggestive for a role played by inflammation in atherosclerosis acceleration. In addition, the evidence that serum and liver tissue proinflammatory markers, such as C reactive protein, IL6, TNF and TGF-β and procoagulant factors, fibrinogen, and plasminogen activator inhibitors are increased in patients with accelerated atherosclerosis reinforces this hypothesis[11]. Furthermore, high-sensitivity C-reactive protein, biomarker of sub-clinical inflammation and of cardiovascular and cerebrovascular disease was found the strongest independent variable associated with NAFLD development in a case-control study[144].

OXIDATIVE STRESS

Several studies have shown that in patients with NAFLD, and in particular in those with NASH, there is evidence of systemic inflammation and increased oxidative stress, relevant factors contributing to metabolic alterations and cardiovascular damage. Altered oxidant/antioxidant status and subclinical inflammation are believed to play a key role in the pathogenesis of atherosclerosis. Hyper-

glycemia and inflammation increase the production of reactive oxygen species (ROS) triggering oxidative stress which is responsible for oxidation of carbohydrates, lipids and protein. Oxidation of polyunsaturated fatty acids gives raise to the production of malonylaldehyde (MDA), 4-hydroxy-nonenal (HNE) and 4-oxy-2-nonenal (ONE). Increases of these markers, have been detected in patients with NAFLD and correlated to vascular damage as well as in animal models and *in vitro* studies[145]. Furthermore, it has been found that specific localization in liver tissues cells of oxidized phosphatidylcholine (oxPC, a lipid peroxide that serves as a ligand for scavenger receptors), suggests that neutrophil myeloperoxidase-derived oxidative stress may be crucial in the progression of steatotic liver disease[146].

In addition inflammation and oxide-reductive imbalance may play an important role in the progression of NAFLD and atherosclerotic disease as documented in patients in chronic hemodialysis in which a progressive chronic liver and vascular damage developed faster than in patients without renal failure[110].

The "oxidative stress theory" implies that the initial phase of atherosclerosis is sustained by oxidation of LDL which accumulate in the arterial wall and are taken up by macrophages which, in turn, transform into foam cells. The increased serum levels of LDL and several other pathways of oxidative stress present in patients with NAFLD will accelerate atherosclerosis process[147]. Interestingly the "iron theory" of atherosclerosis, suggested by Sullivan[148], emphasizes the role of iron as a free radical inducer through oxidative stress could find consistency in patients with NAFLD. In fact the high prevalence of increased ferritin, detected in roughly 30% of the patients, could partly account for the accelerated atherosclerosis of these patients. Recent data point out that the relation between increased ferritin and early atherosclerosis, as evaluated by increased IMT, is valid only in patients negative for HFE mutations, suggesting that patients positive for these mutations with lower level of serum hepcidin may be protected from atherosclerosis[131]. This could explain the apparent paradox of the role of iron in atherosclerosis despite the lack of evidence of increased CVD in patients with hereditary hemochromatosis.

Thus, results from experimental and clinical studies suggest that oxidative stress is increased in patients with NAFLD/NASH and that adipose tissue is very likely the major source of ROS which in turn will cooperate in accelerating atherosclerosis.

ADIPOKYNES IMBALANCE

The increase in adipose tissue and chronic inflammation cause an imbalance in adipokines secretion, in particular a reduction of adiponectin and an increase in leptin, two adipokines with opposite function. Adiponectin has been shown to have anti-inflammatory and anti-fibrotic capability and it is believed that its low level may facilitate the progression of CVD and of steatosis to NASH, in addi-

tion has been reported to have specifically antiatherogenic properties, whereas leptin, found increased in NAFLD, has been shown in animal models to exert opposite effect. Furthermore, leptin has a multifunctional role in inflammation, that is fundamental in the atherogenic pathway, acting as a proinflammatory stimulus. Among other factors released within adipose tissue, TNF-α promotes lipolysis and increases FFAs; both TNF-α and IL-6 are related to mitochondrial and endothelial dysfunction, to atherosclerosis and cardiovascular disease; at the same time the reduction of protective adipokynes as adiponectin improves vascular damage[149-151].

COAGULATION IMBALANCE

Dyslipidemia, lipid-based oxidative injury and necroapoptosis underpin a multifactorial disorder which may result in a hypercoagulable state. This was suggested on the basis of the evidence of long-lasting ongoing inflammatory state associated with this condition and on epidemiological studies, although someone argues that despite an increased CVD risk, no strong evidence has been reported that patients with NAFLD are at increased risk of thrombosis.

Although a direct link between NAFLD and coagulation has not yet established, several cross-sectional studies indicate that NAFLD and NASH are associated with a systemic prothrombotic state possibly through the release of procoagulant factors from steatotic liver (C-reactive protein, plasminogen activator inhibitor-1, fibrinogen), in addition high levels of factors VIII, IX, XII, VII, von Willebrand, soluble tissue factor, platelet hyperaggregability, procoagulant microparticles are found in NAFLD independently of age, BMI waist circumference, plasma triglycerides and insulin resistance[152-155] as well as in cirrhosis[156]. A possible explanation for the failure to ascertain a causal effect between NAFLD and an impairment of coagulation is the lack of appropriate tests. In fact, coagulation evaluated by traditional global tests such as the prothrombin and activated partial thromboplastin times (PT/APTT) or through the measurement of the individual pro- or anti-coagulants does not allow to detect the complex interplay between the procoagulants (*i.e.*, thrombin-generation drivers) and their naturally-occurring anticoagulant counterparts (*i.e.*, thrombin-neutralization drivers) operating *in vivo*. However despite the lack of conclusive data, preliminary results seem to suggest the existence a coagulation unbalance between production and/or half-life of both pro- and anticoagulant proteins[157] as shown in Figure 3.

INCREASED ASSUMPTION OF FRUCTOSE

Patients with NAFLD frequently consume a large amount of fructose, a highly lipogenic sugar which is a common component in all major caloric sweeteners (sucrose, high-fructose corn syrup, honey and fruit juice concentrates) and soft drinks[128,158]. Fructose consump-
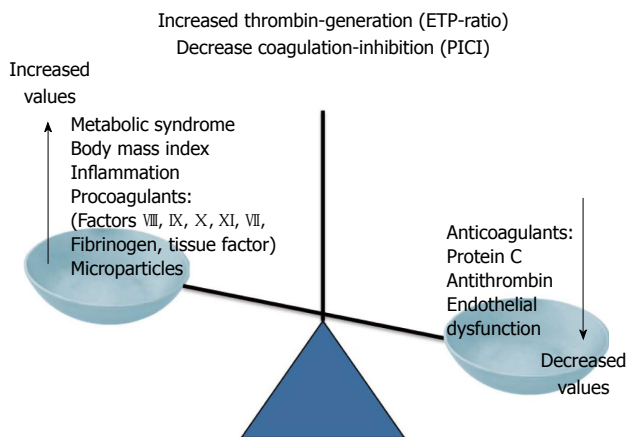
Increased thrombin-generation (ETP-ratio)
Decrease coagulation-inhibition (PICI)

Figure 3 Causes and consequences of the procoagulant imbalance in patients with nonalcoholic fatty liver disease. ETP-ratio: Ratio of the endogenous thrombin potential measured in the presence or absence of thrombomodulin; PICI: Protac®-induced coagulation inhibition.

tion promotes hepatic *de novo* lipogenesis and intrahepatic lipids deposition, inhibition of mitochondrial beta-oxidation of long-chain fatty acids , triglycerides formation and steatosis, increased plasma levels of fasting small dense LDL-C, oxidized LDL-C, and activation of inflammatory pathways. All these fructose-induced effects synergize with the metabolic alterations typical of NAFLD playing a key role in atherosclerosis acceleration. This mechanism is particularly important in lean subjects who very often consume a large amount of fructose.

CONCLUSION

All data reported in this review provide evidence of a strong association between NAFLD and subclinical atherosclerosis. Furthermore, current evidence suggests that NAFLD, although considered the hepatic manifestation of the metabolic syndrome, is emerging as an independent risk factor for the occurrence and progression of CVD. The findings and conclusions are similar in retrospective and prospective studies, which evaluated different manifestations of subclinical atherosclerosis (increased IMT, endothelial dysfunction, arterial stiffness, impaired left ventricular function and coronary calcification).

A general agreement emerging from these studies indicates that patients with NASH are at higher risk of CVD than those with simple steatosis, emphasizing the role of chronic inflammation in the pathogenesis of atherosclerosis of these patients.

It is very likely that the different mechanisms involved in the pathogenesis of atherosclerosis in patients with NAFLD have a different relevance in the different patients according to individual genetic background.

In conclusion, in the presence of NAFLD greater emphasis should be placed on the cardiovascular risk with patients undergoing a complete cardiovascular evaluation to prevent future atherosclerotic complications. Specific life-style modification and aggressive pharmaceutical modification will not only reduce the progression of liver disease, but also reduce morbidity for cardiovascular disease improving over-all prognosis and survival.

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