Research Article

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Epicardial fat, cardiac geometry and cardiac function in patients with non-alcoholic fatty liver disease: Association with the severity of liver disease

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Background & Aims: Non-alcoholic fatty liver disease (NAFLD) has been associated with increased cardiovascular risk, including coronary artery disease and cardiac dysfunction. In addition, recent evidence highlighted the possible role of epicardial fat as a new cardiometabolic risk factor. We tested the correlation between epicardial fat, alterations in cardiac geometry and function, and severity of liver damage, in patients with biopsy-proven NAFLD.

Methods: The anthropometric, biochemical and metabolic features were recorded in 147 consecutive biopsy-proven NAFLD cases (Kleiner score). Epicardial fat thickness was measured by echocardiography.

Results: Epicardial fat was higher in patients with severe *vs.* milder fibrosis (8.5 ± 3.0 *vs.* 7.2 ± 2.3 mm; $p = 0.006$); this association was maintained at multivariate logistic regression analysis (OR 1.22, 95%C.I. 1.01–1.47; $p = 0.04$) after correction for gender, age >50 years, visceral obesity, IFG/diabetes, non-alcoholic steatohepatitis and severe steatosis. Of note, 37.1% of patients with epicardial fat >7 mm (median value) had severe liver fibrosis, compared to 18.3% of the cases with lower epicardial fat ($p = 0.01$). As for echocardiographic indices, after adjusting for cardiometabolic confounders, diastolic posterior-wall thickness ($p = 0.01$), left ventricular mass ($p = 0.03$), relative wall thickness ($p = 0.02$), and left atrial volume (0.04), as well as ejection fraction ($p = 0.004$), lower lateral TDI e′ ($p = 0.009$), E/A ratio (0.04) (cardiac geometry alterations and diastolic dysfunction) were linked to severe liver fibrosis.

Conclusions: In patients with NAFLD, a higher epicardial fat thickness is associated with the severity of liver fibrosis, in keeping with a possible pathogenic role of ectopic fat depots in whole body organ damage. In addition, morphological and functional cardiac alterations are more pronounced according to the severity of fibrosis. Further studies are needed to validate our results.

Keywords: NASH; NAFLD; Epicardial fat; Cardiac dysfunction.
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Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is increasing worldwide, affecting roughly 20–30% of the general population [1]. In addition to an expected risk for disease progression from non-alcoholic steatohepatitis (NASH) to bridging fibrosis, cirrhosis and its complications [1], NAFLD patients are also at higher risk of early asymptomatic cardiovascular alterations and/or frank cardiovascular disease [2]. Specifically, NAFLD, diagnosed either by ultrasonography or by liver biopsy, has been associated with a higher prevalence of low coronary flow reserve [3], coronary calcification [4], and carotid atherosclerosis [5–7], well before the occurrence of cardiovascular events. These alterations have been partly associated with the severity of liver damage, measured by both lobular inflammation and fibrosis. Accordingly, cross sectional studies showed an association between NAFLD and the presence/extent of coronary, cerebral and peripheral cardiovascular involvement [8], whereas longitudinal studies identified NAFLD as a risk factor for incident cardiovascular events after adjustment for cardiometabolic confounders [9].

In the last few years, a number of studies also assessed the association between cardiac morphology or function, and the presence of NAFLD. Specifically, studies in small cohorts of subjects at high [10,11] or low [12] cardiometabolic risk highlighted the association of an ultrasonographic diagnosis of NAFLD, after adjustment for metabolic confounders, with a significant impairment in echocardiographic diastolic function compared to non-NAFLD cases. Along this line, a recent study on a small cohort of NAFLD patients reported significant changes in cardiac structure and function as assessed by MRI, in the absence of metabolic changes or overt cardiac disease [13]. No data were, however, available on the impact of the severity of liver damage on these

cardiac alterations. The complex interplay between liver fat and heart function has been further demonstrated by studies reporting an association between NAFLD and epicardial fat thickness. Epicardial fat thickness, assessed by either magnetic resonance imaging (MRI) [14] or echocardiography [15,16], was higher in NAFLD subjects compared to non-NAFLD. In addition, a correlation was reported between epicardial fat thickness and ALT levels [17], the severity of ultrasonographic (US) [15] or MR spectroscopy [18] steatosis, and the Non-alcoholic Activity Score (NAS) in un-adjusted analyses [16].

In a consecutive cohort of patients with biopsy-proven NAFLD, we assessed whether epicardial fat is correlated to the severity of liver damage, and whether liver damage is linked to cardiac alterations in morphology and function.

Patients and methods

Patients

The study involved 147 consecutive patients with NAFLD, recruited at the Gastrointestinal & Liver Unit of Palermo University Hospital, and fulfilling all the inclusion and exclusion criteria detailed below. Inclusion criteria were: (1) a histological diagnosis of NAFLD on a liver biopsy done less than 6 months before enrollment, showing steatosis (>5% of hepatocytes) with or without necroinflammation and/or fibrosis including cirrhosis. (2) The pre-biopsy assessment of NAFLD was based on chronically elevated ALT for at least 6 months and (3) alcohol consumption of <20 g/day in the year before (also confirmed by a questionnaire). Exclusion criteria were: (1) decompensated cirrhosis (jaundice, presence of ascites or encephalopathy); (2) hepatocellular carcinoma; (3) liver disease of different or mixed etiology (excessive alcohol consumption, hepatitis C, hepatitis B, autoimmune liver disease, Wilson's disease, hemochromatosis, α1-antitrypsin deficiency); (4) human immunodeficiency virus infection; (5) previous treatment with antiviral therapy, immunosuppressive drugs and/or regular use of steatosis-inducing drugs (steroid, amiodarone, tamoxifen, etc.), as assessed at interview; (6) history of heart diseases (both coronary or cardiac disease); (7) active intravenous drug addiction.

The study was carried out in accordance with the principles of the Helsinki Declaration and its appendices, and with local and national laws. Approval was obtained from the hospital's Internal Review Board and its Ethics Committee, and written informed consent was obtained from all patients.

Clinical and laboratory assessment

Clinical and anthropometric data were collected at the time of liver biopsy. Patients were classified as normal weight (BMI 18.5–24.9 kg/m^2), overweight (BMI 25–29.9), obese (BMI ⩾30). Waist circumference (WC) was measured at the midpoint between the lower border of the rib cage and the iliac crest. Visceral obesity was diagnosed in the presence of WC ⩾96 cm in males and ⩾80 cm in females, these thresholds being well applicable to the Mediterranean/European population [19]. The diagnosis of arterial hypertension was based on the following criteria: systolic blood pressure ⩾140 mmHg and/or diastolic blood pressure ⩾90 mmHg (measured three times within 30 min, in the sitting position and using a brachial sphygmomanometer), or use of blood-pressure-lowering agents. The diagnosis of impaired fasting glucose (IFG) and of type 2 diabetes was based on the revised criteria of the American Diabetes Association, using a value of fasting blood glucose ⩾100 to <126, and ⩾126 mg/dl, respectively [20]. In patients with a previous diagnosis of type 2 diabetes, current therapy with insulin or oral hypoglycemic agents was documented.

A 12-h overnight fasting blood sample was drawn at the time of biopsy to determine the serum levels of ALT, total cholesterol, HDL-cholesterol, triglycerides, plasma glucose, insulin, and platelet count. Insulin resistance (IR) was determined according to the homeostasis model assessment (HOMA) method [21], as: Insulin resistance (HOMA-IR) = Fasting insulin (μU/ml) × Fasting glucose (mmol/L)/22.5.

Assessment of histology

Slides were coded and read by one pathologist (D.C.), who was unaware of the patient's identity and history. A minimum length of 15 mm of biopsy specimen or the presence of at least 10 complete portal tracts was required [22]. Steatosis was assessed as the percentage of hepatocytes containing fat droplets (minimum 5%), and evaluated as continuous variable. The Kleiner classification [23] was used to grade steatosis, lobular inflammation, and hepatocellular ballooning, and to stage fibrosis from 0 to 4. NASH was considered to be present when steatosis, ballooning, and lobular inflammation were all present.

Table 1. Demographic, laboratory, metabolic and histological features of 147 consecutive patients with non-alcoholic fatty liver disease.

Variable	Non-alcoholic fatty liver disease (n = 147)
Mean age, years	48 ± 12
Male gender (%)	64
Mean body mass index, kg/m^2	29 ± 4
Body mass index ≥30, kg/m^2 (%)	43
Visceral obesity (%)	85
Alanine aminotransferase, IU/L	74 ± 46
Platelet count, 10^{3}xμU/L	231 ± 69
Cholesterol, mg/dl	202 ± 45
HDL cholesterol, mg/dl	52 ± 17
Triglycerides, mg/dl	136 ± 78
Blood glucose, mg/dl	101 ± 33
Insulin, μU/ml	15 ± 8
HOMA-score	3.73 ± 2.29
IFG/type 2 diabetes (%)	30
Arterial hypertension (%)	35
Smoking (%)	24
Statin use (%)	6
Histology (%)	
Steatosis grade	
1 (5%-33%)	38
2 (>33%-66%)	37
3 (>66%)	25
Lobular inflammation	
0	7
1	52
2	34
3	7
Hepatocellular ballooning	
0	18
1	47
2	30
NASH	76
Stage of fibrosis	
0	21
1	31
2	20
3	18
4	10

Data are given as mean ± SD or as percentage.
HDL, high-density lipoprotein; HOMA, homeostasis model assessment; IFG, impaired fasting glucose; NASH, non-alcoholic steatohepatitis.

Echocardiographic assessment

Within three months from liver biopsy and before starting educational programs, all patients underwent an echocardiography examination by a single experienced observer, using a GE VIVID 7 interfaced with a 1.7/2.4 MHz phased-array probe.