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Circulating Interleukin-6 is a biomarker for coronary atherosclerosis in nonalcoholic fatty liver disease: Results from the Multi-Ethnic Study of Atherosclerosis

Tracey G. Simon [a], Maria Esther Perez Trejo [b], Robyn McClelland [b], Ryan Bradley [c], Michael J. Blaha [d], Irfan Zeb [e], Kathleen E. Corey [a], Matthew J. Budoff [f], Raymond T. Chung [a,*]

[a] Liver Center, Gastrointestinal Division, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States
[b] Department of Biostatistics, University of Washington, Seattle, WA, United States
[c] Division of Preventive Medicine, University of California, San Diego, La Jolla, CA, United States
[d] Johns Hopkins Ciccarone Center for the Prevention of Heart Disease, Baltimore, MD, United States
[e] Department of Cardiology, Mount Sinai St. Luke's Roosevelt Hospital (Bronx-Lebanon Hospital Center), United States
[f] Los Angeles Biomedical Research Institute, Division of Cardiology, Harbor-UCLA Medical Center, Los Angeles, CA, United States

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ABSTRACT

Background: Biomarkers to predict the presence and severity of subclinical cardiovascular disease (CVD) in nonalcoholic fatty liver disease (NAFLD) are lacking.
Methods: 3876 participants from the Multi-Ethnic Study of Atherosclerosis (MESA), without known chronic liver disease underwent baseline non-contrast cardiac CT, with NAFLD defined by validated liver:spleen ratio (L:S) < 1.0, and subclinical CVD defined by coronary artery calcium (CAC) score > 0. Randomly-selected subgroups underwent detailed inflammatory marker testing, including LpPLA2 mass (N = 2951), activity (N = 3020), high-sensitivity C-reactive protein (hsCRP; N = 3849), and interleukin-6 (IL-6; N = 3764). Among those with NAFLD, we estimated the prevalence of CAC > 0 and CAC > 100 for each SD biomarker increase, using multivariable log-binomial regression models adjusted for cardiometabolic risk factors.
Results: Seventeen percent (N = 668) of participants met the criteria for NAFLD. NAFLD participants were younger (mean age 61 ± 10 vs. 63 ± 10 years, p < .0001) but more likely to have an elevated BMI (mean 31.1 ± 5.5 vs. 28.0 ± 5.2 kg/m^2, p < .0001), diabetes (22% vs. 11%, p < .0001), and increased inflammatory biomarkers, including LpPLA2 activity, hsCRP and IL-6 (all p < .0001). Among NAFLD participants, IL-6 was the only biomarker independently associated with prevalent CAC > 0 (PR = 1.06 [1.00–1.11]), or CAC > 100 (PR = 1.09 [1.02–1.17]). In contrast, circulating LpPLA2 mass/activity and hsCRP were not associated with either the prevalence or severity of subclinical CVD (all p > .05).
Conclusion: In a large, multi-ethnic population with NAFLD, IL-6 is independently associated with the prevalence and severity of subclinical atherosclerosis. Further research into the longitudinal effects of NAFLD on progressive CVD will determine whether IL-6 is a marker or mediator of NAFLD-related atherosclerosis.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) represents the leading cause of chronic liver disease in the United States, where it affects approximately 90 million adults [1]. Among those with NAFLD, cardiovascular disease (CVD) is the most common cause of mortality [2], and recent data demonstrate that NAFLD is also associated with the development of atherosclerosis and subclinical CVD [3]. Given that not all patients with NAFLD develop progressive CVD, it has been hypothesized that inflammatory mediators common to the vasculature and the liver may accelerate atherogenesis in certain individuals with NAFLD [4]. However, validated biomarkers for predicting NAFLD-associated atherosclerosis are lacking, and no previous study has examined the relationship between inflammatory markers and subclinical CVD, in a NAFLD population. An improved understanding of CVD risk indicators in NAFLD would allow providers to appropriately identify individuals at highest risk of progressive atherosclerotic disease.

Interleukin-6 (IL-6) is a pleiotropic cytokine that bridges innate and adaptive immunity and serves to regulate the acute-phase response and chronic inflammation [5]. Within the general population, circulating concentrations of IL-6 are associated with subclinical CVD, including endothelial dysfunction, arterial stiffness and coronary atherosclerosis

* Corresponding author at: Liver Center, Division of Gastroenterology, Warren 1007, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, United States.
E-mail address: rtchung@partners.org (R.T. Chung).

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[6,7]. Mendelian randomization studies lend further support to the causal role played by IL-6 in CVD pathogenesis [8,9], and in a recent meta-analysis, each standard deviation (SD) increase in IL-6 corresponded to a 25% increased risk of CVD events in the general population [10].

In contrast, clinical biomarkers of CVD risk in NAFLD remain largely uncharacterized. While preliminary clinical studies demonstrate that plasma inflammatory biomarkers correlate with NAFLD severity [11], to date no published study has examined whether these biomarkers are also associated with atherosclerosis, in NAFLD. The purpose of this study was to evaluate a set of candidate inflammatory markers known to predict CVD within the general population, and to determine their association with the prevalence and severity of coronary atherosclerosis among individuals with NAFLD.

2. Methods

2.1. Study population

The Multi-Ethnic Study of Atherosclerosis (MESA) is a population-based, observational cohort of 6814 Caucasian, African American, Hispanic, and Chinese adults, aged 45–84 years, without known CVD at the time of enrollment. Participants were enrolled at one of six field centers across the United States from July 2000 through September 2002, using a study design that has previously been described [12]. MESA was approved by the institutional review board of each participating site, and all participants provided their written informed consent.

A total of 4389 MESA participants underwent non-contrast cardiac computed tomography (CT) and had adequate imaging for the quantification of liver and spleen fat, using measured attenuation (Hounsfield units). Compared to those excluded for inadequate imaging, the included population was more likely to be older, female, black and Hispanic, with a higher prevalence of obesity, diabetes and hypertension, as has previously been described in MESA [13]. Five hundred thirteen individuals were excluded: 405 had a history of heavy alcohol use (>7 drinks/week in women or >14 drinks/week in men), 59 reported alcohol consumption but did not quantify intake, 39 reported infection with hepatitis B virus or hepatitis C virus, 8 reported cirrhosis and 2 were amiodarone users, leaving a final population of 3876 participants.

2.2. Measurement of IL-6 and other inflammatory biomarkers

Assays for serum inflammatory biomarkers were obtained at baseline from randomly-selected MESA participants in both the main cohort and in selected ancillary studies, as has previously been reported [14]. All samples were obtained following a 12 hour fast. IL-6 was measured in 3764 randomly-selected participants from the full MESA cohort, using ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis MN), with a lower limit of detection of <0.094 pg/mL (range 0.156–10.00 pg/mL), and a 6.3% coefficient of variability. Gamma glutamyltransferase (GGT) activity was measured from frozen samples [15], and high-sensitivity C-reactive protein (hsCRP) was measured by particle-enhanced immunopholometric assay on the BNII nephelometer (Dade-Behring, Inc., Deerfield IL), also from the full MESA cohort [16]. Additional inflammatory biomarkers included soluble intercellular adhesion molecule-1 (sICAM-1), soluble E-selectin, plasminogen activator inhibitor-1 (PAI-1) and lipoprotein associated phospholipase A2 (Lp-PLA2) mass and activity, each of which were measured for an ancillary study, using methods that have previously been described [17,18].

2.3. Outcome ascertainment

Details of the MESA scanning protocol have been reported in detail [19]. All MESA participants underwent unenhanced cardiac CT scans at the baseline examination, with either a cardiac-gated electron-beam CT (Chicago, Los Angeles, New York), or a multidetector CT (Baltimore, Forsyth County, St. Paul). Individuals were scanned twice, with all images interpreted at the MESA CT reading center (Los Angeles Biomedical Research Institute, Torrance CA), where mean CAC (Agatston) score was calculated [20]. For the present study, subclinical atherosclerosis was defined as CAC > 0 (vs. CAC = 0). For the analysis of CAC severity, comparison groups included CAC > 100 vs. CAC = 0.

2.4. Liver fat quantification

Details of the assessment of hepatic steatosis in MESA have previously been reported [21]. Using regions of interest ≥100 mm^2 on baseline cardiac CTs, hepatic and splenic attenuation measurements (in Hounsfield Units) were obtained. The mean of two regions in the right hepatic lobe was divided by the spleen measurement, to calculate the liver:spleen (L:S) attenuation ratio, and hepatic steatosis was defined by L:S < 1.0 [21].

2.5. Assessment of covariates

MESA collected clinical and socio-demographic variables, including age, sex, race and ethnicity, behavioral risk factors including smoking status (current, former or prior smoking and total pack-years) and alcohol consumption (number of drinks/day) [12]. Participants also reported detailed medical histories at baseline, including diabetes, cardiovascular conditions, cancer, and regular physical activity, estimated by metabolic equivalent minutes (MET-MIN) per week [12]. Anthropomorphic measurements including waist circumference (centimeters) and body mass index (BMI, kg/m^2) were obtained from trained examiners. Obesity was defined as BMI ≥30 kg/m^2. Using an automated sphygmomanometer (Critikon, Tampa FL), systolic and diastolic blood pressure measurements were obtained three times, and the mean of the last two measurements was used. Diabetes was defined as a self-reported physician diagnosis of diabetes, diabetic medication use, or a fasting glucose ≥126 mg/dL. Serum glucose was assayed by a hexokinase/glucose-6-phosphate dehydrogenase method, and triglycerides and cholesterol were assayed by enzymatic methods following a 12-hour fast. HDL cholesterol (HDL-C) was measured after precipitation of non-HDL-C with magnesium/dextran and low-density lipoprotein cholesterol (LDL-C) was calculated via the Friedewald equation. Lipid-lowering medication use was defined as use of prescribed statins, ezetimibe, fibrates, niacin and/or other lipid-lowering medications; in this cohort, as in the MESA cohort as a whole, >90% of those taking any lipid-lowering medication were taking statins. Metabolic syndrome was defined by the American Heart Association/National Heart, Lung and Blood Institute criteria [22].

2.6. Statistical analysis

Baseline demographic and clinical characteristics were compared in individuals with and without NAFLD using descriptive statistics and frequency distributions. Levels of inflammatory biomarkers were calculated by mean (±SD) and median [interquartile range, IQR]. Among those with NAFLD (N = 668), we tested correlations between log-transformed biomarker concentrations using Pearson's correlation. We also tested the relationship between continuous levels of each biomarker and cardiometabolic risk factors chosen a priori for their association with subclinical CVD (i.e. age, sex, ethnicity, BMI, waist circumference, triglycerides, HDL-C, dyslipidemia, HOMA-IR, alcohol intake and smoking status), using univariable linear regression, in which regression coefficients and P-values were generated by regressing each clinical factor on the biomarker of interest.

We constructed a series of multivariable log-binomial regression models to estimate the prevalence of [1] CAC > 0 vs. CAC = 0, and [2] CAC > 100 vs. CAC = 0, for each one SD increase in biomarker. The following multivariable models were constructed: Model 1, adjusted for age (years), sex, ethnicity and MESA site; Model 2, adjusted for Model 1 + smoking status (current, former, never) and servings/day of alcohol; Model 3, adjusted for Model 2 + BMI (kg/m^2) and diabetes; Model 4, adjusted for Model 3 + systolic blood pressure, use of anti-hypertensive medications, total cholesterol, HDL cholesterol, use of lipid-lowering medications, and regular physical activity (MET-MIN/week). Multiplicative interactions between the inflammatory biomarkers, age and ethnicity were assessed in the fully-adjusted adjusted models.

Due to the known relationship between IL-6 and both visceral adiposity and insulin resistance [23], we constructed two additional models that further accounted for (a) waist circumference (Model 5), or (b) HOMA-IR (Model 6). Finally, due to the observed correlation between IL-6 and high-sensitivity C-reactive protein (hsCRP), we constructed a final model that also adjusted for continuous hsCRP, in addition to traditional cardiovascular risk factors (Model 7). All P-values were two-tailed and a P < .05 was considered statistically significant. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

3. Results

Among 3876 eligible participants, 668 (17.2%) had CT evidence of NAFLD (L:S < 1.0). Baseline clinical and demographic characteristics are presented according to NAFLD status (Table 1). Participants with NAFLD were younger (mean age 61 ± 10 vs. 63 ± 10 years, P < .0001), with a larger mean BMI (31.2 ± 5.5 vs. 28.0 ± 5.2 kg/m^2, P < .0001) and more likely to be Hispanic (35.8% vs. 20.5%, P < .0001), with diabetes (22% vs. 11%, P < .0001) and hypertension (53% vs. 46%, P < .0001). However, there was no significant difference in diagnosed dyslipidemia or in use of lipid-lowering medications, when NAFLD vs. non-NAFLD participants were compared (both P = .338).

3.1. Inflammatory biomarkers in NAFLD and non-NAFLD participants

Table 2 outlines the sample size (N) and the mean (SD) and median [IQR] concentration for each biomarker, in individuals with and without NAFLD. Overall, NAFLD participants had increased median [IQR] inflammatory biomarker concentrations, including soluble ICAM-1, PAI-1, hsCRP and IL-6 (all P < .0001; Table 2).