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Original article

Interleukin-10 as a predictor of major adverse cardiovascular events in a racially and ethnically diverse population: Multi-Ethnic Study of Atherosclerosis

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ABSTRACT

Purpose: To understand if baseline levels of the anti-inflammatory cytokine interleukin-10 (IL-10) are associated with either subclinical atherosclerosis or risk for adverse cardiovascular (CV) events.
Methods: The study included 930 adults from the Multi-Ethnic Study of Atherosclerosis (MESA) ancillary Stress Study. Participants, age 48–90 years at enrollment, were followed for an average of 10.2 years. IL-10 level was measured at the initial Stress Study visit. Cardiovascular outcomes were defined as composite CV death, myocardial infarction, stroke, stroke death, and resuscitated cardiac arrest. Coronary calcification was determined by Agatston coronary artery calcium (CAC) score. The association between IL-10 level and CV event risk was evaluated by Cox proportional hazard modeling, while that of IL-10 level and CAC presence and amount was determined with prevalence risk ratio (PRR) and linear regression modeling, respectively. Models were adjusted for CV risk factors and proinflammatory biomarkers.
Results: After full adjustment, IL-10 level did not predict CV events (HR 1.19, 95%CI 0.89, 1.60) and was not associated with CAC prevalence (PRR 1.00, 95%CI 0.94, 1.07), nor amount of CAC in those with nonzero CAC (β -0.01, 95%CI -0.23, 0.21).
Conclusion: In individuals without clinical heart disease, baseline IL-10 level appears unrelated to risk of CV events and is a poor marker of subclinical coronary atherosclerosis.

Introduction

Interleukin-10 (IL-10) is an anti-inflammatory cytokine thought to be protective against the development and progression of atherosclerosis. Secreted by macrophages, B cells, and T-helper cells in response to systemic inflammation, IL-10 suppresses antigen-presenting capacity, dendritic cell activity, and T-cell proliferation, as well as negatively regulates proinflammatory cytokine production [1, 2]. With respect to cardiovascular (CV) disease, animal data suggest that IL-10 prevents atherosclerotic plaque development [3, 4], improves plaque stability [3], and promotes lesion size reduction [5].

Given the anti-inflammatory and anti-atherosclerotic properties of IL-10, higher levels would be expected to protect against CV disease and events. Indeed, data suggest that a higher level of IL-10 at the time of an acute coronary syndrome event is protective against risk for future cardiovascular events [6–8]. Yet, epidemiologic data do not consistently demonstrate this relationship. In clinically stable populations, preliminary evidence from two studies suggests that higher IL-10 concentrations relate to increased, as opposed to decreased, risk for adverse cardiovascular events [9, 10]. Importantly, these results may be condition specific, as the two cohorts were of 1) older adults [10] and 2) postmenopausal women with known cardiovascular disease [9]. A possible explanation for these counterintuitive findings is that IL-10, produced in response to circulating cytokines such as

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interleukin-6 (IL-6) and tumor necrosis factor α (TNFα) [11, 12], acts as a surrogate marker of an overall proinflammatory milieu. However, the relationship between proinflammatory and anti-inflammatory cytokines in individuals free of clinical heart disease, as well as the context in which steady-state IL-10 levels relate to cardiovascular disease development require further exploration.

Designed to explore the role of stress and inflammation in CV disease, the Multi-Ethnic Study of Atherosclerosis (MESA) Stress Study provides a unique opportunity to study IL-10 and its role in CV risk prediction. The Stress Study cohort is a community-dwelling, racially, and ethnically diverse population from whom both traditional cardiovascular risk factors, as well as a broader selection of inflammatory biomarkers were collected. Utilizing this robust data set, we examined the relationship of IL-10 level to the overall inflammatory milieu, as well as the ability of IL-10 level to predict future adverse cardiovascular events and the prevalence and amount of coronary artery calcium (CAC). We hypothesized that IL-10 level is positively associated with proinflammatory biomarkers, and that higher a IL-10 level would increase risk for future CV events as well as be positively associated with prevalence and amount of coronary artery calcification.

Methods

Study population

MESA is a prospective, longitudinal cohort study designed to understand risk factors related to the development and progression of CV disease and incident CV events. MESA recruited 6814 men and women aged 45–84 years free from overt CV disease at baseline from six communities including: Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles, California; New York, New York; and St. Paul, Minnesota. Initial examination occurred between July 2000 and August 2002 with longitudinal follow-up through July 2015. Detailed information regarding MESA study design and recruitment has been published previously [13]. The Institutional Review Boards of all participating institutions approved the study protocols and consent procedures.

The data utilized in this study come from the MESA Stress Study, an ancillary study investigating the role of stress in cardiovascular disease. The Stress Study enrolled a cohort of 1002 MESA participants at either MESA visit 3 or visit 4 via the New York, NY, and Los Angeles, CA, field center between July 2004 and November 2006. About 500 individuals were enrolled at each site, resulting in a random sample at each site of black, Hispanic, and white participants. Of note, because the MESA New York field center did not enroll Asians, Stress Study enrollment was limited to white, black, and Hispanic individuals. Beyond the standard MESA data collection, inflammatory biomarker data, including IL-6, TNFα, and IL-10, were collected from participants. Additional details of the Stress Study have been published previously [14–17].

Our objective was to explore the relationship between proinflammatory and anti-inflammatory biomarkers in individuals free of clinical heart disease, as well as the relationship between IL-10 level, risk of future CV events, and CAC prevalence and amount. We therefore excluded 53 individuals with incomplete inflammatory biomarker profiles. We excluded an additional 19 individuals who experienced a cardiovascular event before inflammatory biomarker data collection, leaving a total initial sample of 930 participants.

Biomarker measurement

Most of the physiological and laboratory data used in the analyses were collected at the Stress Study visit as described previously [17]. Briefly, biomarkers including IL-10, IL-6, TNFα, and high sensitivity C-reactive protein (hsCRP) were collected during a morning blood draw from fasting individuals. Cytokine assays were performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). Concentrations of IL-6, IL-10, and TNFα were measured by enzyme-linked immunosorbent assay (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN), milliplex MAP human cardiovascular disease panel 3 (Millipore Corporation; Billerica, MA), and LINCOplex Human Cardiovascular Disease Panel 3 kit (Millipore Corporation, St. Charles, MO), respectively. The average analytical coefficient of variability for IL-6, IL-10, and TNFα was 6.3%, 8.1%, and 10.3%, respectively. Levels of hsCRP were only available from MESA visit 1. Despite the temporal discordance, this biomarker was incorporated into the analysis given the previously described relationship between elevated hsCRP and the risk for cardiovascular disease [18]. The level of hsCRP was measured using immunonephelometry (N hsCRP; Dade Behring Inc, Deerfield, IL, USA) with an interassay coefficient of variability of 5.7% [19].

Additional covariates

Additional covariates were determined from Stress Study visit data. Data include age, gender, race/ethnicity (white, black, or Hispanic), body mass index (BMI), systolic blood pressure (SBP), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), aspirin use, statin use, antihypertensive medication use, diabetes (present or absent), and smoking status (never, prior, or current smoker). Resting blood pressure was measured using the automated oscillometric method (Dinamap). Three measurements were obtained while individuals were in the seated position; the average of the last two measures was used for analyses. Fasting TC and HDL were measured in plasma using a cholesterol esterase, cholesterol oxidase reaction (Chol R1, Roche Diagnostics) in individuals who had fasted more than or equal to 10 hours. Diabetes status was determined according to the American Diabetes Association definition of fasting glucose more than 126 mg per dL or a history of medical management of diabetes [20]. Smoking status, current aspirin use, current statin use, and current antihypertensive medication use were determined by self-report.

Outcomes

CV events

Cardiovascular events were defined as time to first CV event, which was a composite outcome of cardiovascular death, myocardial infarction, and stroke, stroke death, and resuscitated cardiac arrest. Events were identified by participant self-report as well as review and abstraction of medical records, including death certificates. Events were adjudicated by at least two physicians, with differences of opinion resolved by committee review. Data on incident CV events are available through the follow-up end date of December 31, 2015. Follow-up time was measured from the date of the MESA Stress exam (corresponding to either MESA visit 3 or 4) up to the date of the first CV event (for those who experienced an event) or last date of follow-up.

Coronary artery calcium measurement

MESA participants underwent two coronary artery computed tomography (CT) scans to assess coronary artery calcification, as described previously [21]. The initial scan for each participant occurred during MESA visit 1. Each participant had one follow-up coronary artery CT scan that occurred either at MESA visit 2 or visit 3. For this study, the Agatston CAC score obtained closest to the participant's Stress Study visit (either visit 3 or 4) was used. In

individuals with nonzero CAC, the log-transformed Agatston score was used as a measure of total amount of coronary calcification. The time between coronary artery CT and Stress study visit was measured in years.

Analysis

All inflammatory biomarkers were winsorized at the first and 99th percentile and then log-transformed before parametric analysis. Pearson correlation analyses between inflammatory biomarkers IL-10, IL-6, TNFα, and hsCRP, and Spearmen correlation analyses between IL-10 and patient characteristics were performed. Kaplan–Meier time-to-event analysis of the unadjusted association between IL-10 levels and time to major adverse cardiovascular events was performed; estimates were compared using the log-rank test. The association between IL-10 level and CV event risk was evaluated by Cox proportional hazard modeling. Three models were used: Model 1 was unadjusted; Model 2 was adjusted for proinflammatory biomarkers IL-6, TNFα, and hsCRP; Model 3 was adjusted for proinflammatory biomarkers as well as common CV risk factors including age, gender, race/ethnicity, BMI, SBP, TC, HDL cholesterol, aspirin use, statin use, any antihypertensive medication use, diabetes status, and smoking status. Additional regression models were fit to determine the association between IL-10 level and CAC. Given that the frequency of CAC = 0 versus CAC greater than 0 is near 50% in the MESA population [22], prevalence risk ratio (PRR) regression modeling was utilized in favor of logistic regression to determine the relationship between IL-10 level and the presence or absence of CAC. PRR regression assumed Gaussian error and used robust standard error estimates. In individuals with nonzero CAC, linear regression models were fit to evaluate the relationship between IL-10 level and amount of CAC. Three models were used: Model 1 was adjusted for time from coronary CT scan to Stress Study visit; Model 2 was adjusted for time from coronary CT to Stress Study visit as well as proinflammatory biomarkers IL-6, TNFα, and hsCRP; Model 3 was adjusted for time from coronary CT to Stress Study visit, proinflammatory biomarkers, and the common CV risk factors of age, gender, race/ethnicity, BMI, SBP, TC, HDL cholesterol, aspirin use, statin use, any antihypertensive medication use, diabetes status, and smoking status.

Results

Baseline characteristics and summary statistics for inflammatory biomarkers of the 930 individuals from the MESA Stress Study are provided in Table 1. The average follow-up time was 10.2 years (±2.6) and the number of major adverse CV events was 83 (8.9%). We found that the level of the anti-inflammatory cytokine IL-10 is weakly correlated with levels of proinflammatory biomarkers IL-6 ($r = 0.086$, $P < .01$) and TNFα ($r = 0.097$, $P < .01$) and has no correlation with hsCRP (Table 2). Kaplan–Meier time-to-event analysis of unadjusted associations between IL-10 levels and time to major adverse cardiovascular event demonstrated no significant differences between IL-10 level quartiles and outcomes (Fig. 1). In the unadjusted Cox proportional hazard models, one SD increase in log IL-10 concentration showed a trend toward association with major adverse CV events (HR 1.26, 95% CI 0.97–1.65; $P = .09$) (Table 3). After adjusting for proinflammatory biomarkers, as well as other covariates known to influence CV risk, the hazard ratio was not statistically significant (HR 1.19; CI 0.89–1.60; $P = .24$) (Table 3). With respect to CAC, in all models, IL-10 level was not related to the estimated prevalence risk for CAC nor was IL-10 level associated with total amount of CAC in individuals with nonzero CAC (Table 4). Of note, despite the lack of significance with respect to IL-10, the proinflammatory biomarkers demonstrated significant association with major adverse CV events and CAC. In Cox proportional hazard modeling, IL-6 level was significantly associated with adverse CV events in Model 2 (HR 1.70, 95% CI 1.16–2.49; $P < .01$), although this relationship did not remain significant in the fully adjusted Model 3 (data not shown). Moreover, IL-6 (Model 2: PRR 1.2, 95% CI 1.08–1.33, $P < .001$) and TNFα (Model 2: PRR 1.19, 95% CI 1.11–1.29, $P < .0001$; Model 3: PRR 1.12, 95% 1.05–1.20, $P < .001$) levels were positively associated with the presence of CAC (data not tabulated), while IL-6 level was also positively associated with the amount of CAC in Model 2 (β 0.43; 95% CI 0.12–0.74, $P < .01$), although not in the fully adjusted model (data not tabulated).

Table 1
Baseline characteristics of MESA Stress ancillary study cohort

Variable ($n = 930$)	N (%) or mean (SD.)
Age (range 48–90)	65 (9.8)
Men	434 (47%)
Race/ethnicity	
White	179 (19%)
Black	267 (29%)
Hispanic	484 (52%)
BMI (kg/m^2)	29 (5.7)
Diabetic	162 (17%)
Smoking status	
Never	429 (46.4%)
Former	310 (33.6%)
Current	183 (20.0%)
Systolic blood pressure (mm Hg)	123.3 (20.4)
Total cholesterol (mg/dL)	190.8 (38.1)
HDL cholesterol (mg/dL)	51.4 (15.1)
Medication use	
Aspirin at least 3 d per wk	272 (29%)
Statin	203 (22%)
Any antihypertensive medication	426 (46.8%)
Follow-up time (y)	10.2 (2.6)
Incidence of major adverse CVD events	83 (8.9%)
Coronary calcification (Agatston)*	
CAC = 0	453 (48.7%)
CAC < 100	270 (29.0%)
CAC > 100	207 (22.3%)
Time between Coronary CT and Stress Study visit (y)	1.5 (1.2)
Inflammatory markers	
IL-10 (pg/mL)	7.98 (13.34)
IL-6 (pg/mL)	2.83 (1.94)
TNFα (pg/mL)	4.32 (5.74)
hsCRP (mg/L)†	3.85 (5.20)

BMI = body mass index; HDL = high-density lipoprotein cholesterol; CAC = coronary artery calcium; IL-10 = interleukin-10; IL-6 = interleukin-6; TNFα = tumor necrosis factor α; hsCRP = high sensitivity C-reactive protein.
* Agatston coronary artery calcium (CAC) score obtained closest to the Stress Study visit.
† The average time between hsCRP measurement (MESA visit 1) to other inflammatory biomarker measurement (Stress Study visit) was 3.86 ± 0.9 years.

Discussion

In this prospective, longitudinal study of a racially and ethnically diverse population, we set out to characterize the relationship between proinflammatory and anti-inflammatory cytokines, as well

Table 2
Correlation between IL-10 and proinflammatory biomarker levels

	IL-6	TNFα	hsCRP
IL-10	0.086*	0.097*	−0.009

IL-10 = interleukin-10; IL-6 = interleukin-6; TNFα = tumor necrosis factor α; hsCRP = high sensitivity C-reactive protein.
Data presented as Pearson correlation coefficients.
Biomarkers were log transformed before analysis.
* $P \leq .01$.

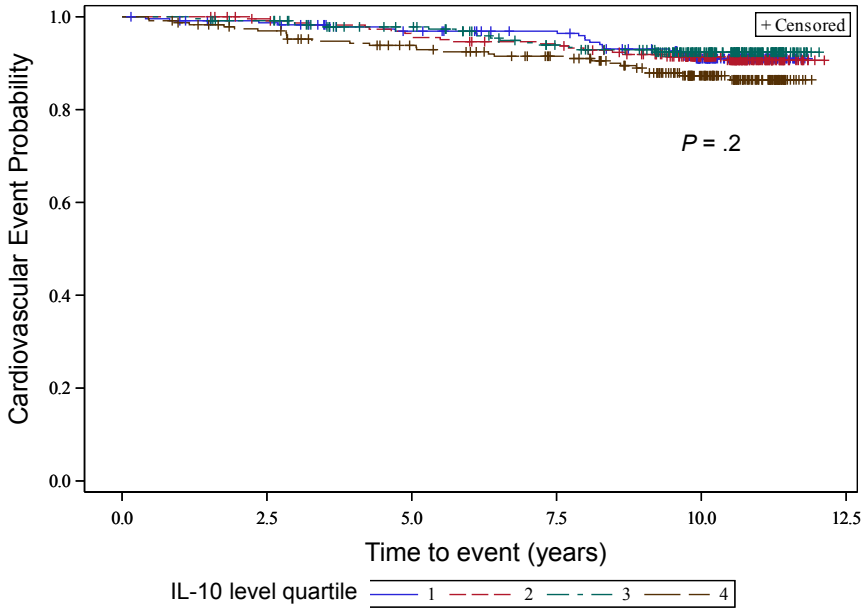

Fig. 1. –Time to major adverse cardiovascular event by IL-10 level. IL-10 levels are grouped from lowest (quartile 1) to highest (quartile 4). There is no significant difference between IL-10 level quartiles and outcome ($P = .2$).

as confirm the association between the anti-inflammatory cytokine IL-10 and risk for major adverse cardiovascular events. We confirmed the hypothesis that IL-10 level is positively correlated with the levels of some proinflammatory biomarkers, albeit weakly. However, we were unable to confirm a relationship between IL-10 level and risk for future major adverse cardiovascular events, presence or absence of coronary calcification, nor the total amount of coronary calcification.

The relationship between circulating proinflammatory and anti-inflammatory cytokines remains incompletely characterized. Data suggest that IL-10 level increases simultaneously with other inflammatory cytokines, including IL-6 and TNFα, during an acute coronary event [23, 24], leading to the hypothesis that IL-10 is a surrogate marker of the overall inflammatory milieu. Although we found levels of IL-6 and TNFα to be positively related to IL-10, the association was weak. Moreover, with the exception of gender, TC, and HDL cholesterol, we were unable to find a significant association between IL-10 concentration and other patient characteristics that may influence inflammation such as obesity, diabetes, statin or aspirin use, or smoking status (Supplementary Table 1). Therefore, although IL-10 level may represent a surrogate marker of the overall inflammatory milieu during an acute event, we were unable to confirm a strong relationship in a population without clinically significant cardiac disease at baseline. The interrelation between IL-10 level and the steady-state inflammatory milieu requires further investigation.

Table 3
Cox proportional hazard models of adverse CV events by IL-10 level

Model	Hazard ratio* (95% CI)	*P*
Model 1 ($n = 930$)	1.26 (0.97–1.65)	.09
Model 2 ($n = 930$)	1.21 (0.92–1.58)	.17
Model 3 ($n = 901$)	1.19 (0.89–1.60)	.24

CI = confidence interval; IL-10 = interleukin-10; SD = standard deviation.
Model 1—Unadjusted model.
Model 2—Adjusted for proinflammatory biomarkers IL-6, TNFα, and hsCRP.
Model 3—Adjusted for proinflammatory biomarkers with additional adjustment for age, gender, race/ethnicity, BMI, total cholesterol, HDL, smoking status, diabetes status, aspirin use, statin use, and any antihypertensive medication use.
All biomarkers were log transformed before analysis.
* Hazard ratio of adverse CV event by SD change in log-transformed IL-10 level.

Evidence from epidemiologic studies suggests that the predictive ability of IL-10 for fatal and nonfatal cardiovascular events is highly context dependent. The Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) cohort was composed of older men and women (age 70–82 years) from Scotland, Ireland, and the Netherlands [10]. In this large group of close to 6000 individuals, baseline levels of IL-10 were associated with a slightly increased risk for adverse events, a finding that was specific to individuals without preexisting vascular disease [10]. On the other hand, in the much smaller Estrogen Replacement and Atherosclerosis (ERA) study of 309 postmenopausal women, IL-10 concentration was positively correlated with adverse outcomes in women with coronary artery disease [9]. Taken together, these data potentially support a role for IL-10 as a risk predictor in distinct population subsets. In this study, we investigated whether risk associated with IL-10 elevation could be more broadly applied to a contemporary, diverse, community-dwelling population without clinically significant cardiovascular disease at baseline. Contrary to the previous studies, we do not find a relationship between IL-10 level and adverse cardiovascular events (Table 3). The wide variability between cohorts with respect to age, racial/ethnic, and gender differences likely contributes to the lack of consistent findings. Although IL-10 may be a useful biomarker within specific populations, its utility for cardiovascular risk prediction in the general population remains questionable.

Small studies suggest that higher levels of IL-10 are associated with lower coronary artery atherosclerotic burden [25–27] and more plaque stability [28], leading to the hypothesis that IL-10 may slow atherosclerotic disease development and progression. However, we did not find a relationship between IL10 level and the presence or absence of coronary artery calcification. In addition, we confirm a previous finding that steady-state levels of IL-10 are not associated with total coronary calcification amount [29]. Although IL-10 may impact atherosclerosis in a local vascular-bed environment, systemic levels of IL-10 appear unrelated to coronary artery disease severity.

Table 4
Association of IL-10 levels with prevalence and amount of coronary artery calcification

IL-10 association with prevalence of CAC	Prevalence risk ratio of CAC* (95% CI)	*P*
Model 1 ($n = 930$)	1.06 (0.97–1.14)	.16
Model 2 ($n = 930$)	1.02 (0.94–1.10)	.65
Model 3 ($n = 909$)	0.99 (0.93–1.06)	.88
IL-10 association with amount of CAC in individuals with CAC > 0	**Mean difference in CAC† (95% CI)**	***P***
Model 1 ($n = 477$)	0.01 (−0.06 to 0.35)	.93
Model 2 ($n = 477$)	−0.05 (−0.25 to 0.22)	.89
Model 3 ($n = 462$)	−0.01 (−0.23 to 0.21)	.94

CAC = coronary artery calcium; CI = confidence interval; IL-10 = interleukin-10; SD = standard deviation.
Model 1—Adjusted for time between CAC measurement and Stress Study visit.
Model 2—Adjusted for time between CAC measurement and Stress Study visit as well as for proinflammatory biomarkers IL6, TNFα, and hsCRP.
Model 3—Adjusted for time between CAC measurement and Stress Study visit and proinflammatory biomarkers with additional adjustment for age, gender, race/ethnicity, BMI, total cholesterol, HDL, smoking status, diabetes status, aspirin use, statin use, and any antihypertensive medication use.
All biomarkers were log-transformed before analyses.
Agatston score was log-transformed before analyses.
* Prevalence risk ratio of CAC by SD increase in log-transformed IL-10 level.
† Mean difference in log-transformed Agatston score by SD increase in log-transformed IL-10 level.

This study has a number of potential limitations. The first concern is that only a small number of proinflammatory cytokines were utilized to determine the overall inflammatory environment. Indeed, it is likely that some components of inflammation were not captured by the available biomarkers. However, the weak relationship between IL-10 level and the proinflammatory markers is consistent with findings in other studies of clinically stable populations [10]. Moreover, the data presented here that proinflammatory biomarkers are associated with adverse CV outcomes and coronary calcification suggest that the inflammation captured in this cohort is similar to previous studies [30–34]. The second limitation is the variable amount of time between coronary CT and the Stress Study visit. To minimize the impact of this temporal discrepancy, the results presented here adjust for the time between imaging and biomarker measurement when appropriate. Finally, the small number of cardiovascular events observed in the MESA Stress Study precluded age- and race/ethnicity-stratified analyses to look for group-specific associations between IL-10, CV events, and presence of coronary artery disease. Future analysis may be undertaken once more events have accumulated in this population.

In conclusion, in this racially and ethnically diverse population of individuals free of clinical heart disease at baseline, we were able to demonstrate a weak association between circulating levels of IL-10 with proinflammatory cytokines. However, we were unable to confirm previous reports that the steady-state IL-10 concentration relates to risk for future CVD events, nor is it associated with the presence or amount of coronary calcification. Although the anti-inflammatory properties of IL-10 with respect to CV disease are intriguing, consistent results in large epidemiologic studies implicating IL-10 in risk prediction remain elusive. Additional work aimed at understanding the complexity of cytokine interrelationships, as well as the molecular mechanisms with which IL-10 affects plaque architecture and stability, may help with future studies. However, at this time, the utility of IL-10 in clinical risk prediction models remains limited.

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Supplementary Table 1
Correlation of patient characteristics with log-transformed IL-10 level

Age	−0.037
Gender	0.087†
Race/ethnicity	0.045
BMI	0.024
Diabetes	0.035
Smoking status	0.007
Systolic blood pressure	−0.037
Total cholesterol	−0.151**
HDL cholesterol	−0.183**
Aspirin use	0.0577
Statin use	0.051
Any antihypertensive medication use	0.067*

IL-10 = interleukin-10; BMI = body mass index; HDL = high-density lipoprotein. Data presented as Spearman correlation coefficients. IL-10 concentration was log-transformed before analysis; *$P \leq .05$; †$P \leq .01$, **$P \leq .001$.