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Cross-sectional Relations of Multiple Inflammatory Biomarkers to Peripheral Arterial Disease: The Framingham Offspring Study

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Abstract

Background—Emerging evidence suggests that different inflammatory biomarkers operate through distinct biologic mechanisms. We hypothesized that the relation to peripheral arterial disease (PAD) varies for individual markers.

Methods—In a community-based sample we measured 12 biomarkers including *plasma* CD40 ligand, fibrinogen, lipoprotein-associated phospholipase-A2 mass and activity, osteoprotegerin, P-selectin, and tumor necrosis factor receptor 2 (TNFR2); and *serum* C-reactive protein, intracellular adhesion molecule-1, interleukin-6, monocyte chemoattractant protein-1, and myeloperoxidase in Framingham Offspring Study participants (n=2800, 53% women, mean age 61 years). We examined the cross-sectional relation of the biomarker panel to PAD using 1) a global test of significance to determine whether at least one of 12 biomarkers was related to PAD using the TEST statement in the LOGISTIC procedure in SAS and 2) stepwise multivariable logistic regression with forward selection of markers with separate models for 1) ankle-brachial index (ABI) category (<0.9, 0.9 to 1.0, >1.0) and 2) presence of clinical PAD (intermittent claudication or lower extremity revascularization).

Results—The group of inflammatory biomarkers were significantly related to both ABI and clinical PAD (p= 0.01 and p= 0.02, respectively, multi-marker adjusted global significance test). Multivariable forward elimination regression retained interleukin-6 and TNFR2 as significantly associated with PAD. For one standard deviation change in interleukin-6 and TNFR2 concentrations, there was a 1.21 (p=0.005) and 1.19 (p=0.009) increased odds of a change in ABI level respectively. Similar results were observed for clinical PAD.

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Conclusion—Interleukin-6 and TNFR2 were significantly associated with PAD independent of established risk factors and each other, suggesting that each marker represents a distinct biologic pathway.

Keywords

peripheral arterial disease; ankle-brachial index; interleukin-6; tumor necrosis factor receptor 2

Introduction

Peripheral arterial disease (PAD) affects approximately five to eight million Americans (1) and is a powerful predictor of incident coronary heart disease, stroke, and mortality. (2–4) The ankle-brachial index (ABI), a subclinical measure of PAD, is considered a marker of generalized atherosclerosis. It is now well established that inflammation plays a central role in the pathogenesis of atherosclerosis and, further, that various inflammatory markers predict incident cardiovascular disease (CVD) events. (5;6) However, risk for CVD associated with inflammatory markers is often attenuated with adjustment for traditional risk factors.

The relation between inflammatory markers and PAD is not fully characterized. C-reactive protein predicts risk of symptomatic PAD, (7;8) and also is associated with atherosclerosis in the aorta (9) and femoral artery (10), but the associations are attenuated after accounting for established risk factors. Adjusted inverse relations between the ABI and C-reactive protein have been reported in men (11), in ever smokers (12), and in persons with prevalent CVD (13). Higher C-reactive protein concentrations are associated with progression of aortic, iliac, and lower extremity atherosclerosis (14), and in one small study both a low ABI and a high C-reactive protein identified persons at greatest risk for clinical events and death. (15) Reports of the relations between other inflammatory markers and PAD are limited, often focus on a single marker, or on small hospital-based or referral-based samples, and demonstrate conflicting results.(10;16–18) Emerging evidence suggests that different inflammatory markers operate through distinct biologic mechanisms, and thus the relative importance to the atherosclerotic process and PAD may differ for individual markers.

We examined the cross-sectional relations of a panel of 12 inflammatory biomarkers and PAD in a large community-based sample. We selected the inflammatory and oxidative stress markers to represent various stages and pathways in the inflammatory process, including chemokines (monocyte chemoattractant-1), cytokines (interleukin-6, tumor necrosis factor-alpha and tumor necrosis receptor-2; Selectins [P-selectin and CF40 ligand] cell adhesion molecules [intercellular adhesion molecule-1]) acute phase reactants (C-reactive protein, fibrinogen), and an oxidative stress marker (myeloperoxidase). We hypothesized that different inflammatory markers represent distinct biologic pathways, and thus not all markers would be related to PAD. Moreover, we postulated that the strength of the relation would vary for different biomarkers operating through unique pathways. To our knowledge, no other study has evaluated the relation between multiple biomarkers from potentially diverse biologic pathways and PAD conjointly.

METHODS

Study Sample

The Framingham Offspring Study was initiated in 1971 when 5124 adult children (and offspring spouses) of the Original cohort were enrolled in the Framingham Heart Study. Offspring participants have been examined approximately every 4 to 8 years since the study's inception. Written informed consent was obtained at each examination and the Institutional Review Board of Boston University Medical Center approved the examination content.

Offspring participants who attended the seventh examination cycle (1998 to 2001) were eligible for this study. The examination included a standardized medical history and physical examination, electrocardiogram, noninvasive cardiovascular testing, and measurement of fasting lipids, glucose, and a panel of inflammatory biomarkers. Of the 3539 participants attending the examination, 205 were examined off-site and did not have ABI testing, 92 participants had incomplete ABI data, and 12 participants were excluded because of an ABI >1.4. We further excluded participants missing all biomarker data (n=289) and participants with incomplete risk factor data (n=141). Thus, our study sample included 2800 Offspring participants with data available for all 12 biomarkers and complete risk factor data.

Measurement of Ankle-brachial index

Ankle-brachial systolic blood pressure measurements were obtained using a standard protocol by trained technicians and the details previously published.(19) An 8 megahertz Doppler pen probe and an ultrasonic Doppler flow detector (Parks Medical Electronics, Inc.) were used to measure the systolic blood pressure in each limb. All limb blood pressures were repeated, and if the initial and repeat blood pressures differed by more than 10 mmHg at any one site, a third measurement was obtained. Measurements were obtained from the dorsalis pedis artery only if the posterior tibial pulse could not be located by palpation or with Doppler probe. For this study, the ABI was defined as the ratio of the average systolic blood pressure in the ankle divided by the average systolic blood pressure in the higher arm. The lower ABI was used for analysis. Based on prior epidemiologic studies, we analyzed ABI <0.9 as indicative of PAD.

Intermittent Claudication and Lower Extremity Revascularization

Intermittent claudication was assessed using a standardized physician-administered questionnaire that inquired about the presence of exertional calf discomfort related to walking uphill or walking rapidly and was relieved with rest. Two physicians independently interviewed all participants suspected to have intermittent claudication. An endpoint panel, comprised of three senior investigators, examined all medical evidence and made the final diagnosis of the presence of intermittent claudication. Participants were also queried about revascularization procedures including lower extremity bypass surgery and percutaneous transluminal angioplasty. The endpoint panel reviewed hospital records for all cardiovascular procedures.

Inflammatory Biomarker Measurement

At examination cycle seven, 12 biomarkers were measured including *plasma* CD40 ligand, fibrinogen, lipoprotein-associated phospholipase A2 mass and activity, osteoprotegerin, P-selectin, and tumor necrosis factor receptor 2 (TNFR2); and *serum* C-reactive protein, intracellular adhesion molecule-1, interleukin-6, monocyte chemoattractant protein-1, and myeloperoxidase. Specimens were collected from fasting participants and plasma and serum aliquots were stored at −80 °C until analysis. Biomarkers, except C-reactive protein, were measured in duplicate with commercially available ELISA kits from R&D Systems (intracellular adhesion molecule-1, interleukin-6, monocyte chemoattractant protein-1, P-selectin, TNFR2), Bender MedSystems (CD40 ligand), Diagnostica (fibrinogen), Oxis (myeloperoxidase) and ALPCO (osteoprotegerin). The Dade Behring BN100 nephelometer was used to measure high sensitivity C-reactive protein. Lipoprotein-associated phospholipase A2 activity was measured by GlaxoSmithKline, and mass was measured by DiaDexus. Details for assays have been previously published (20). The intra-assay coefficients of variation for the biomarkers were as follows: CD40 ligand 4.4±3.4%, fibrinogen 1.1±1.1%, intracellular adhesion molecule-1 3.7±2.4%, interleukin-6 3.1±2.1%, lipoprotein-associated phospholipase A2 activity 7.0% (low) and 5.9% (high) and mass (based on 24% duplicate readings) 6% (low) and 8% (high) concentrations, monocyte chemoattractant protein-1 3.8±3.3%, myeloperoxidase 3.0±2.5%, osteoprotegerin 3.7±2.9%, P-selectin 3.0±2.2%, TNFR2 2.2

±1.6%. The kappa statistic based on 146 C-reactive protein samples was 0.95. Additionally, plasma tumor necrosis factor alpha (R&D Systems, CV 7.6% low, 5.6% high control) and urinary isoprostanes, 8-Epi-$PGF_{2\alpha}$ (Cayman, Ann Arbor, MI; CV 9.1±5.8%), indexed to urinary creatinine were measured on a subset of participants.

Clinical Covariate Assessment

Covariates were defined at the time of examination cycle seven. Medication use and current smoking within the year preceding the exam were self-reported. Resting blood pressure was measured twice by the examining physician. Hypertension was defined as an average blood pressure of systolic ≥140 or diastolic ≥90 mm/Hg or use of anti-hypertensive medication. Body mass index was calculated as weight in kilograms divided by the height in meters squared. Diabetes was defined by fasting blood glucose of ≥126 mg/dL, or use of insulin or oral hypoglycemic agents. CVD was defined as coronary heart disease, stroke or transient ischemic attack, and heart failure. An endpoint adjudication panel made the final diagnostic determination using previously reported criteria.(21)

Statistical Analysis

Sex-specific standardization of biomarkers was performed (i.e., within each sex, biomarkers were standardized to have a mean of 0 and a standard deviation of 1). Due to skewed distributions, biomarker concentrations were natural logarithmically transformed for analysis. Our primary analysis was the simultaneous consideration of multiple biomarkers (independent variables) in relation to PAD defined as two separate variables: 1) ABI category (ABI: <0.9, 0.9–1.0, >1.0) and 2) presence of clinically overt PAD defined as intermittent claudication or lower extremity revascularization. Separate logistic regression models were run for ABI category and presence of clinically overt PAD. First, we performed a global test of significance to determine whether at least one of 12 biomarkers was related to the PAD dependent variables using the TEST statement in the LOGISTIC procedure in SAS. The analysis was adjusted for age, sex, and the following 13 clinical covariates previously reported to be correlated with biomarkers and or PAD (19;22;23): current cigarette smoking, number of pack-years of cigarette smoking, diabetes, fasting glucose, body mass index, waist circumference, total to HDL cholesterol ratio, fasting triglyceride, lipid lowering treatment, hypertension, aspirin use, prevalent CVD (myocardial infarction, coronary insufficiency, angina pectoris, stroke, or transient ischemic attack), and use of hormone replacement therapy. Second, we conducted a stepwise multivariable ordinal logistic regression with PAD as the dependent variable, with forward selection of biomarkers using a $p<0.05$ adjusting for age, sex, and forcing the 13 clinical covariates into the model. For biomarkers identified to be related to PAD in the second step of the analysis we calculated point estimates of the odds ratio (or i.e., the relative change in odds of PAD), with 95% confidence intervals, per standard deviation increase of the biomarker examined.

We conducted several secondary analyses. We examined effect modification by age (<60, ≥60 years) and sex for significant biomarker—PAD relations. We repeated the analysis in persons free of CVD. Finally because multiple reports have used different markers or sets of markers, we analyzed the multivariable-adjusted linear relations of each log-transformed marker (dependent variable), one marker at a time, to the independent PAD measures using PROC GLM in SAS. Tumor necrosis factor-alpha was measured on a subset of participants attending examination cycle seven (n=2129) and was included in the secondary analysis. SAS version 8.1 was used to perform all analyses.(24)

RESULTS

Participant Characteristics and Biomarker Concentrations

Clinical characteristics of the study sample by presence of clinically overt PAD, and by ABI category are shown in Table 1. Participants with PAD, defined by symptoms or an ABI <0.9, were older than participants without PAD. The untransformed median for the 12 biomarkers and tumor necrosis factor-alpha (available on a subset) by ABI level are shown in Table 2. A graded increase in marker concentrations across decreasing ABI levels was present for all markers except lipoprotein-associated phospholipase A2 mass and activity whereas an inverse relation was seen for CD40 ligand.

Global relations of multiple biomarkers and measures of PAD

The inflammatory markers as a group were significantly related to both ABI and clinically detected PAD (p=0.01 and p=0.02 respectively for the multi-marker adjusted global test of significance) as shown in Table 3. The forward elimination regression retained interleukin-6 and TNFR2 in the final models as significantly associated with ABI and with intermittent claudication or lower extremity revascularization. The odds of a one category reduction in ABI level increased by 21% and 19% per a one-standard deviation increase in interleukin-6 and TNFR2, respectively. Similar results were observed for intermittent claudication or revascularization.

Secondary Analyses

In analyses of ABI, excluding participants with prevalent CVD (n=2496), the global test examining whether the markers as a group were related to ABI was not significant (p=0.34). The forward stepwise selection regression retained only interleukin-6 with a nearly identical point estimate (estimate 1.21, 95% confidence interval 1.05, 1.39, p=0.01). The analysis of clinical PAD was not run in participants free of prevalent CVD due to small numbers (n=43). No significant interactions were noted for sex and age with regard to the association between biomarkers and ABI.

In adjusted regression models examining each marker separately, C-reactive protein, interleukin-6, fibrinogen, tumor necrosis factor alpha, and TNFR2 were significantly inversely related to ABI level (p-values ranging from <0.0001 to 0.02). For each biomarker, we exponentiated the adjusted mean log-transformed biomarker and its 95% confidence interval to obtain its adjusted geometric mean and corresponding 95% confidence interval (Table 4). Similar markers were associated with clinical PAD (C-reactive protein, interleukin-6, and TNFR2; p-values ranging from < 0.0001 to 0.01) with the following exceptions: fibrinogen and tumor necrosis factor alpha were not significantly associated (data not shown).

DISCUSSION

Principal Findings

In our cross-sectional community-based study, we examined the relations of a panel of 12 inflammatory biomarkers to PAD assessed by ABI, and by clinically defined intermittent claudication and/or lower extremity revascularization. Interleukin-6 and TNFR2 were significantly related to both measures of PAD. In secondary analyses, examining the relation of each marker separately to ABI, we observed additional significant inverse relations for C-reactive protein, fibrinogen, and tumor necrosis factor alpha after adjusting for known risk factors.

Interleukin-6 and PAD

Interleukin-6 is known to play a critical role in the inflammatory process with both pro-inflammatory and anti-inflammatory effects that include the stimulation of C-reactive protein, fibrinogen and other acute phase reactants and increased endothelial cell adhesiveness. In accordance with our results, in a small study of patients with intermittent claudication, interleukin-6 concentrations were higher in patients compared to healthy controls both at rest and after treadmill exercise ($p < 0.001$) suggesting that this marker is associated with peripheral atherosclerosis (25). In a hospital-based investigation of the interleukin-6 G (−174) C genotypes, in patients with type II diabetes with and without PAD, the GG genotype and higher plasma concentrations of interleukin-6 and other inflammatory markers were more common in PAD patients (26). The investigators of that report hypothesize that the GG genotype promotes PAD in patients with diabetes by inducing release of interleukin-6 which in turn results in increased concentrations of other biomarkers such as C-reactive protein. In the Edinburgh Artery Study, inflammatory marker concentrations, including interleukin-6, were significantly elevated at baseline in participants who developed symptomatic PAD during follow-up (27). In that study, interleukin-6 was a predictor of incident PAD but the association was attenuated with adjustment for CVD risk factors.

Elevated concentrations of interleukin-6 have been noted in a community-based sample of older participants with a low ABI (28), a finding similar to our study. Furthermore, interleukin-6 was predictive of PAD progression defined by declining ABI over 12 years of follow up even after adjusting for traditional risk factors and other inflammatory markers (C-reactive protein, intracellular adhesion molecule-1, vascular adhesion molecule-1, and E-selectin) (16), and hemostatic factors. (17) Moreover, interleukin-6 was the only inflammatory marker independently associated with ABI decline in persons free of baseline PAD. The independent predictive value of interleukin-6 in relation to PAD progression may reflect its role in both inflammatory and hemostatic processes. Additionally, interleukin-6 predicts the development of type II diabetes (29) and hypertension (30), both significant predictors of PAD. Finally, interleukin-6 predicts risk for incident CVD events (5) and persons with coronary disease have nearly a threefold risk of intermittent claudication. Hence, the association between interleukin-6 and PAD is likely mediated through a variety of complex inter-related biologic pathways and appears to extend to early peripheral atherosclerosis, atherosclerosis progression, and incident symptomatic disease.

TNFR2 and PAD

Tumor necrosis factor alpha is a pro-inflammatory cytokine that affects vascular tissues including endothelial cells. Tumor necrosis factor alpha exerts its biologic effects through two cell surface receptors, TNFR1 and TNFR2. However, the role of TNFR2 in the regulation of inflammatory responses in endothelial cells is unclear. In mice, the proatherogenic effect of tumor necrosis factor alpha was mediated primarily through TNFR2. (31) Further, in mice endothelial TNFR2 is essential for tumor necrosis factor alpha induced leukocyte-endothelial-cell interaction which mediates several important steps of the inflammatory response including leukocyte rolling, adhesion, and transmigration.(32) A potential mechanism for TNFR2 mediated endothelial dysfunction is the down regulation of lysyl oxidase, a key enzyme in extracellular matrix maturation. TNFR2 has been shown to be involved in lysyl oxidase down-regulation, which in turn is associated with endothelial dysfunction. (33) To our knowledge there is only one small study of patients with intermittent claudication and critical limb ischemia demonstrating elevated tumor necrosis factor receptor concentrations compared with controls. (34)

Other markers and PAD

In the Physician's Health Study, C-reactive protein was the strongest nonlipid predictor of the development of symptomatic PAD (8). In that report both C-reactive protein and fibrinogen improved risk prediction for PAD. However, the two markers were correlated and C-reactive protein was the stronger predictor of risk. The associations between C-reactive protein and fibrinogen and incident PAD were confirmed by the Edinburgh Artery Study and persisted after accounting for risk factors and prevalent CVD (27). Additional associations between C-reactive protein and ABI, PAD progression, and risk for adverse CVD events among individuals with PAD have been reported (11;13;28). However, these prior reports were limited as only a few other biomarkers were examined. If we considered each marker separately, both C-reactive protein and fibrinogen were associated with PAD. But in our global model that considered all 12 biomarkers conjointly neither C-reactive protein nor fibrinogen was significantly associated with PAD. One possible explanation may be that the effect of C-reactive protein and fibrinogen may be mediated through interleukin-6 and TNFR2. It is known that interleukin-6 up-regulates both C-reactive protein and fibrinogen and that all three biomarkers are correlated.

Strengths and Limitations

The strengths of the present study include the community-based sample, the simultaneous measurement of a panel of biomarkers, and the direct measurement of clinical factors previously reported to be correlated with PAD and or the inflammatory markers. Several limitations merit comment. The study is cross-sectional and thus we cannot infer that the associations between PAD and the inflammatory markers are causal. We suspect, but cannot establish with the current study design that the relations are bidirectional, with inflammation contributing to PAD and PAD exacerbating systemic inflammation. Conversely, we note that we may have failed to detect small to modest associations. Medication usage (aspirin and lipid lowering treatments) may have altered some inflammatory marker concentrations. Since medication usage was higher in those with PAD, our results may have been biased toward a null result. In addition, the estimated effect sizes of the observed associations were modest; we acknowledge that statistical significance is not synonymous with clinical significance. We acknowledge that walk test data would have enhanced the accuracy of a PAD diagnosis. Lastly, our sample is primarily white, limiting the ability to generalize our results to other racial and ethnic groups.

Conclusions

In a community-based sample interleukin-6 and TNFR2 were significantly associated with PAD accounting for established risk factors. Their effects appear to be independent of each other suggesting that each marker represents a distinct biologic pathway mediating the complex process of vascular inflammation in peripheral atherosclerosis. Further research is needed to establish the role of these markers in predicting incident clinical PAD events and disease progression and to determine whether therapies targeting these markers alter prognosis in patients with PAD.

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Table 1

Clinical Characteristics of the Study Sample

Variable Mean (SD) or percent	Intermittent claudication		Ankle-brachial Index		
	Yes or Vascular Intervention N=90	No N=2710	<0.9 N=111	0.9 to 1.0 N=225	>1.0 to 1.4 N=2464
Age, years	67 (9)	61 (9)	70 (8)	65 (10)	60 (9)
Women, %	43	54	50	71	52
Current smoking, %	27	13	30	26	12
Pack years among ever smokers, mean	63 (25)	43 (23)	65 (25)	47 (23)	41 (22)
Diabetes, %	29	12	26	20	11
Body mass index, kg/m^2	29.7 (6.1)	28.0 (5.1)	28.2 (5.5)	28.5 (6.6)	28.0 (4.9)
Total/HDL cholesterol ratio	4.5 (1.4)	4.0 (1.3)	4.4 (1.5)	4.0 (1.5)	4.1 (1.3)
Lipid lowering treatment, %	49	19	40	28	19
Hypertension, %	74	44	76	62	42
Hypertension treatment, %	68	32	66	49	31
Aspirin use*, %	57	30	49	34	30
Prevalent cardiovascular disease†, %	52	10	33	15	9
Hormone replacement among women, %	38	30	16	31	31

*aspirin use is defined as 3 or more tablets per week

†cardiovascular disease did not include intermittent claudication

Table 2

Unadjusted Inflammatory Marker Data by Ankle-Brachial Index Level

	Ankle-brachial index		
Marker, units	**<0.9 N=111**	**0.9 to 1.0 N=225**	**>1.0 to 1.4 N=2464**
	Untransformed Marker Concentrations, Median (lower, upper quartile)		
CD40 ligand, ng/mL	0.78 (0.46, 2.31)	0.96 (0.53, 3.04)	1.27 (0.56, 4.07)
C-reactive protein, mg/L	3.77 (1.99, 8.89)	3.71 (1.53, 7.08)	2.02 (0.94, 4.76)
Fibrinogen, mg/dL	425 (384, 481)	388 (349, 446)	368 (326, 418)
Intercellular adhesion molecule-1, ng/mL	283 (243, 323)	255 (223, 292)	239 (209, 279)
Interleukin-6,pg/mL	4.82 (2.85, 7.84)	3.46 (2.21, 5.66)	2.58 (1.75, 4.09)
$LpPLA_2$, mass, nmol/mL/min	284 (231, 374)	293 (231, 367)	288 (229, 360)
$LpPLA_2$, activity, ng/mL	143 (120, 173)	134 (116, 162)	141 (119, 165)
Monocyte chemoattractant protein-1, pg/mL	346 (283, 412)	326 (269, 409)	310 (252, 378)
Myeloperoxidase, ng/mL	43.4 (29.5, 61.7)	40.3 (26.9, 58.2)	39.9 (27.8, 59.6)
Osteoprotegerin, pmol/L	6.71 (5.35, 8.36)	5.85 (4.88, 7.10)	5.30 (4.39, 6.34)
P-selectin, pg/mL	39.7 (31.4, 54.0)	38.5 (31.4, 48.7)	36.0 (28.2, 45.1)
Tumor necrosis factor receptor 2, pg/mL	2407 (2024, 3187)	2154 (1718, 2856)	1945 (1642, 2340)
Tumor necrosis factor alpha*, pg/mL	1.51 (1.15, 1.85)	1.37 (0.99, 1.92)	1.18 (0.92, 1.58)
Urine* 8-epi-$PGF_{2\alpha}$, ng/mmol	162 (103, 247)	151 (100, 234)	131 (88, 192)

*TNF-α data is available on a subset of 2129 participants, urine 8-epi-PGF_2 available on 2404 participants LpPLA2= lipoprotein-associated phospholipase A2

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Table 3

Joint Consideration of Biomarkers in Relation to the Ankle-brachial Index and Clinical Peripheral Arterial Disease

	Global P*	Stepwise Selection Biomarker†	Odds Ratio (95% Confidence Interval)‡	*p*-value
ABI¶	0.01	Interleukin-6	1.21 (1.06, 1.38)	0.005
		TNFR2	1.19 (1.05, 1.36)	0.009
Intermittent Claudication or Lower	0.02	Interleukin-6	1.36 (1.06, 1.74)	0.02
Extremity Revascularization		TNFR2	1.31 (1.04, 1.64)	0.02

*A simultaneous test of whether at least one of the 12 biomarkers were related to PAD (PAD is the dependent variable).

Covariates in multivariable model include age, sex, current cigarette smoking, number of pack-years of cigarette smoking, diabetes, fasting glucose, body mass index, waist circumference, total to HDL cholesterol ratio, fasting triglyceride, lipid lowering treatment, hypertension, aspirin use (≥3 per week), prevalent cardiovascular disease (excluding intermittent claudication), and hormone replacement therapy use (women only).

†Individual biomarkers significantly related to PAD after forward stepwise selection (PAD is the dependent variable) are displayed.

‡Point estimate indicates relative change in odds of PAD (ABI level or presence versus absence of intermittent claudication or lower extremity revascularization) per 1-standard deviation increment in log-marker (1-standard deviation increment is 0.71 for log Interleukin-6 and 0.30 for log TNFR2).

¶The ABI was categorized as follows: <0.9, 0.9 to 1.0, >1.0.

Table 4

Secondary analyses: Multivariable-adjusted Regression of Individual Biomarkers on Ankle-brachial Index*

	Geometric Means and 95% Confidence Intervals† Ankle-brachial index Level			
Biomarker	**<0.9**	**0.9 to 1.0**	**>1.0 to 1.4**	***p*-value**
C-reactive protein	2.91 (2.42, 3.51)	2.44 (2.15, 2.77)	2.19 (2.11, 2.28)	0.007
Interleukin-6	3.64 (3.21, 4.13)	3.09 (2.84, 3.37)	2.86 (2.78, 2.93)	0.0005
Fibrinogen	394 (381, 407)	372 (363, 381)	372 (369, 374)	0.005
Tumor necrosis factor alpha	1.43 (1.29, 1.58)	1.32 (1.24, 1.42)	1.25 (1.22, 1.27)	0.02
TNFR2	2258 (2142, 2379)	2087 (2013, 2164)	2009 (1987, 2030)	<0.0001

* Biomarkers with P<0.05 displayed.

† For each biomarker (dependent variable), we exponentiated the adjusted mean log-transformed biomarker and its 95% confidence interval to obtain its adjusted geometric mean and corresponding 95% confidence interval. Covariates in multivariable model include covariates listed in Table 3 legend.