

Relation of Inflammation to Peripheral Arterial Disease in the National Health and Nutrition Examination Survey, 1999–2002

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The relation between inflammation and peripheral arterial disease (PAD) is not well characterized. This study examined this relation and its consistency across important subgroups in a cross-sectional, nationally representative sample of the adult United States population. C-reactive protein (CRP), fibrinogen, leukocyte count, and PAD were assessed in a sample of 4,787 participants aged ≥ 40 years in the National Health and Nutrition Examination Survey 1999–2002. PAD was defined as an ankle-brachial blood pressure index < 0.9 . Graded relations were present between inflammatory markers and PAD. The multivariate adjusted odds ratios of PAD associated with the highest versus the lowest quartile of CRP, fibrinogen, and leukocyte count were 2.14 (95% confidence interval [CI] 1.41 to 3.25), 2.49 (95% CI 1.27 to 4.85), and 1.67 (95% CI 0.84 to 3.31), respectively (each p trend < 0.05 across quartiles). Associations between inflammation and PAD were similar across gender, obesity, and diabetic subgroups. However, the odds ratios of PAD for the highest CRP quartile versus the 3 lowest quartiles were 3.10 (95% CI 1.76 to 5.45) for non-Hispanic blacks versus 1.50 (95% CI 0.98 to 2.28) for non-Hispanic whites and 1.11 (95% CI 0.57 to 2.17) for Mexican Americans (p interaction = 0.049) and 5.59 (95% CI 1.82 to 17.17) for patients aged 40 to 54 years versus 2.01 (95% CI 1.13 to 3.58) for patients aged 55 to 69 years and 0.98 (95% CI 0.65 to 1.48) for patients aged ≥ 70 years (p interaction = 0.018). Odds ratios of PAD for the highest fibrinogen quartile versus the lowest 3 quartiles were 3.26 (95% CI 1.69 to 6.28) for current smokers versus 0.83 (95% CI 0.51 to 1.35) for never smokers (p interaction = 0.006). In conclusion, in the general United States adult population, inflammation is independently associated with PAD. © 2005 Elsevier Inc. All rights reserved. (Am J Cardiol 2005;96:1579–1583)

Whether inflammation is associated with peripheral arterial disease (PAD) has not been clearly established. In addition, previous studies that examined this association did not examine differences in the association between inflammation and PAD across important subgroups. The purpose of the present study was twofold: (1) to assess the relation among 3 measures of inflammation (C-reactive protein [CRP], fibrinogen, and leukocyte count) and PAD in a representative sample of the general adult United States population and (2) to examine the consistency of the association between inflammation and PAD across subgroups defined by age, gender, race/ethnicity, cigarette smoking, obesity, and diabetes.

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Methods

Study participants: The National Health and Nutrition Examination Survey (NHANES) 1999–2002 was a nationally representative sample of the United States noninstitutionalized, civilian population. As described in detail on the Web site of the National Center for Health Statistics,¹ NHANES 1999–2002 included the oversampling of non-Hispanic blacks and Mexican Americans to provide stable estimates for these groups. Ankle-brachial index (ABI) measurements were obtained on the subsample of patients aged ≥ 40 years.

ABI measurement and PAD definition: For patients with ≥ 1 arm and weighing ≤ 400 pounds, supine systolic blood pressure was measured with blood pressure cuffs on the right brachial artery and the 2 posterior tibial arteries. For patients aged 40 to 59 years, 2 measurements were taken at each site and averaged, and for patients aged ≥ 60 years, 1 measure was taken at each site. For patients with conditions precluding the measurement of the right arm, left brachial artery systolic blood pressure was taken ($n = 22$). Left and right ABI values were calculated as the ratio of left and right ankle systolic blood pressure, respectively, to arm systolic blood pressure. The smallest of the left and right ABI measurements was used. Patients with ABIs ≥ 1.5 ($n = 10$)

Table 1
Comparison of study covariables and inflammatory markers by ABI category

Variable*	ABI				p Trend
	<0.9	0.9–1.06	1.07–1.15	≥1.16	
Population distribution	4.9 ± 0.3%	26.7 ± 0.9%	33.3 ± 0.9%	35.2 ± 1.4%	
Age (yrs)	68.9 ± 0.8	58.9 ± 0.5	54.8 ± 0.3	53.7 ± 0.3%	<0.001
Women	57.9 ± 3.6%	66.3 ± 1.5%	53.2 ± 2.0%	38.2 ± 1.8%	<0.001
Race/ethnicity					
Non-Hispanic white	79.9 ± 2.3%	72.9 ± 2.1%	76.6 ± 2.3%	82.3 ± 1.8%	
Non-Hispanic black	13.7 ± 2.5%	12.4 ± 1.6%	8.7 ± 1.3%	4.9 ± 0.6%	<0.001
Mexican American	3.1 ± 0.8%	4.0 ± 0.8%	4.7 ± 0.7%	5.1 ± 0.9%	
Less than high school education	38.3 ± 2.8%	25.6 ± 1.6%	20.5 ± 1.5%	17.0 ± 1.5%	<0.001
Smoking					
Former	41.5 ± 3.2%	29.2 ± 1.4%	32.7 ± 1.7%	34.3 ± 1.6%	<0.001
Current	28.1 ± 2.1%	25.6 ± 1.7%	21.4 ± 1.2%	15.1 ± 1.3%	
Physical inactivity	59.4 ± 3.0%	42.8 ± 1.7%	37.6 ± 2.6%	28.2 ± 2.0%	<0.001
Diabetes	19.4 ± 2.4%	9.3 ± 1.1%	8.3 ± 0.8%	7.5 ± 0.7%	<0.001
Hypertension	73.6 ± 3.0%	51.6 ± 1.7%	38.7 ± 1.6%	31.7 ± 1.6%	<0.001
Coronary heart disease	29.0 ± 2.9%	11.3 ± 0.9%	9.5 ± 0.8%	7.8 ± 0.7%	<0.001
Stroke	10.1 ± 1.8%	4.4 ± 0.8%	2.2 ± 0.5%	1.9 ± 0.5%	<0.001
Systolic BP (mm Hg)	143.3 ± 1.8	134.0 ± 0.7	127.3 ± 0.7	123.7 ± 0.7	<0.001
Diastolic BP (mm Hg)	69.7 ± 0.9	74.0 ± 0.5	75.2 ± 0.4	74.7 ± 0.5	<0.001
Body mass index (kg/m ²)	27.5 ± 0.4	28.1 ± 0.2	28.2 ± 0.3	28.3 ± 0.2	0.104
Waist girth (cm)	99.0 ± 1.2	96.9 ± 0.6	97.4 ± 0.7	98.8 ± 0.6	0.194
Total cholesterol (mg/dl)	213.6 ± 3.5	215.1 ± 1.2	213.1 ± 1.9	208.5 ± 1.8	0.024
CRP (mg/dl) [†]	0.38 ± 0.34–0.44	0.27 ± 0.24–0.30	0.22 ± 0.20–0.24	0.18 ± 0.17–0.19	<0.001
Fibrinogen (mg/dl) [†]	411.6 ± 399.4–419.9	372.4 ± 365.0–379.9	350.7 ± 343.8–361.4	340.4 ± 333.6–347.2	<0.001
Leukocyte count (SI) [†]	7.4 ± 7.0–7.8	7.0 ± 6.8–7.1	6.8 ± 6.6–6.9	6.6 ± 6.5–6.7	<0.001

* Values are expressed as mean ± SE or percentage ± SE, unless otherwise indicated.

[†] Values are expressed as geometric mean (95% confidence interval).

BP = blood pressure.

are expected to have severe arterial rigidity and were excluded from the current analyses. For these analyses, PAD was defined as ABI <0.9.

Covariate and inflammatory marker measurement:

Age; gender; race/ethnicity; smoking status; level of education achieved; physical activity; and history of diabetes, congestive heart failure, angina, heart attack, and stroke were assessed by self-report. Patients who had not smoked ≥100 cigarettes in their lifetimes were considered never smokers; patients who had smoked ≥100 cigarettes in their lifetimes were considered former smokers if they answered negatively to the question “Do you smoke now?” and current smokers if they answered affirmatively. Patients were considered physically inactive if they reported not participating in any moderate, vigorous, or muscle-strengthening activities in the preceding 30 days. A history of coronary heart disease was considered to be present if the participant reported having been told by a doctor that he or she had congestive heart failure, coronary heart disease, angina, or a heart attack. Seated systolic and diastolic blood pressures were measured separately from ABI testing using a mercury sphygmomanometer according to the American Heart Association’s recommendations.² Up to 3 measurements were averaged for systolic and diastolic pressures. Patients were considered hypertensive if they reported current blood pressure-reducing medication use and/or had systolic blood

pressures ≥140 mm Hg and/or diastolic blood pressures ≥90 mm Hg. Body mass index was calculated as weight in kilograms divided by height in meters squared. Total cholesterol was measured enzymatically.^{3,4} High-sensitivity CRP was measured by latex-enhanced nephelometry, fibrinogen by the Clauss clotting method, and leukocyte count by the Beckman Coulter method of counting using VCS technology (Beckman Coulter, Inc., Fullerton, California).^{3,4}

Statistical methods: Of the 5,849 NHANES 1999–2002 participants aged ≥40 years, 792 were excluded for missing ABI measurements, 10 were excluded for ABI measurements ≥1.5, and an additional 260 were excluded for missing inflammatory marker data. This left 4,787 patients (2,445 men and 2,342 women) for the current analyses. To explore associations across the range of ABI levels, ABI was categorized into 4 levels, with <0.9 (patients with PAD) as the lowest level, and tertiles of ABI for patients without PAD (i.e., ABI ≥0.9). Mean values for continuous study variables and percentages for categorical study variables were calculated by ABI categories. Assessments of trends across ABI categories were made using the median ABI value for each category, via linear and logistic regression. The distributions of inflammatory markers were highly skewed. Therefore, inflammatory markers were divided into quartiles for the remaining analyses. The mean ABI was calculated by quartile of each inflammatory marker after

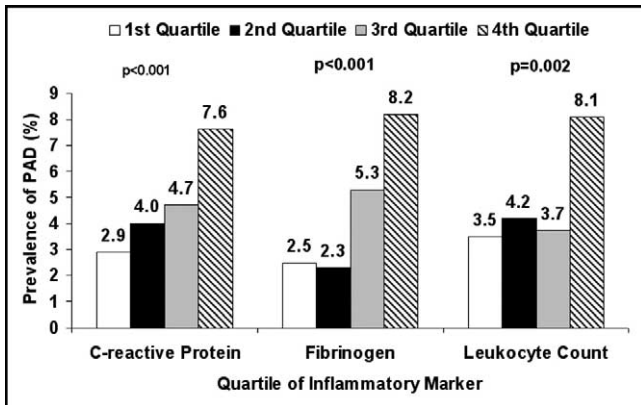


Figure 1. Age-standardized prevalence of PAD (ABI <0.9) by quartile of inflammatory markers for adults aged ≥ 40 years.

adjustment for important covariates using linear regression. The frequency of PAD (ABI <0.9) was calculated by quartile of each inflammatory marker, and differences across quartiles were assessed with chi-square tests. The odds ratios of PAD associated with the highest 3 quartiles of each inflammatory marker were calculated using logistic regression, with the lowest quartile serving as the reference, after adjustment for important covariates. The assessment of a linear trend in odds ratios of PAD across quartiles of inflammatory markers was performed by modeling each inflammatory marker as a 4-level variable, whereby each level was set to the median value of the inflammatory marker in that quartile. Next, analyses were stratified by gender, race and ethnicity, smoking status, age, obesity, and diabetes. For these analyses, inflammatory markers were dichotomized as the highest quartile versus the lowest 3 quartiles pooled together. The formal testing of interactions was performed in the full sample with logistic regression models that included multiplicative interaction terms. All analyses were conducted using SUDAAN 9.0 (Research Triangle Institute, Research Triangle Park, North Carolina) and used techniques appropriate to the complex survey design of NHANES 1999–2002.

Results

The mean ABI was 1.10 (SE 0.003), and the prevalence of PAD (ABI <0.9) was 4.90% (SE 0.33). Patients in lower ABI categories were more likely to have a number of traditional cardiovascular disease risk factors (Table 1). Additionally, geometric mean CRP, fibrinogen, and leukocyte count were incrementally greater with each successively lower ABI category.

Graded, positive associations were present between quartiles of all 3 inflammatory markers and PAD prevalence (Figure 1). Associations remained present after adjustment for traditional cardiovascular disease risk factors (Table 2). These associations were consistent when ABI was treated as

a continuous variable. After adjustment for age, gender, race/ethnicity, education, smoking status, diabetes, physical inactivity, total cholesterol levels, body mass index, and systolic blood pressure levels, mean ABI levels were 1.12, 1.11, 1.10, and 1.09, respectively, across the lowest to the highest quartiles of CRP; 1.12, 1.12, 1.10, and 1.08, respectively, across the lowest to the highest quartiles of fibrinogen; and 1.12, 1.11, 1.11, and 1.09, respectively, across the lowest to the highest quartiles of leukocyte count (each p trend <0.001).

The association between inflammation and PAD did not differ significantly by gender, obesity status, or diabetes status (Table 3). However, the magnitude of the association between inflammation and PAD significantly varied across race/ethnicity, smoking status, and age groupings. The association between CRP and PAD was stronger in non-Hispanic blacks; a similar, although nonsignificant, difference across racial/ethnic groups was also present for fibrinogen. The associations of CRP and fibrinogen with PAD were incrementally stronger in former and current smokers compared with never smokers. An incremental pattern was also seen with age, whereby the association between inflammation and PAD was weaker in older age groups.

Discussion

These data provide evidence of strong, graded, positive associations between inflammation and PAD. Even after adjustment for traditional cardiovascular disease risk factors, patients in the highest quartile of CRP, fibrinogen, or leukocyte count had approximately 2 times greater odds of having PAD than those in the lowest quartile. Additionally, these data showed inflammation to maintain a stronger association with PAD in non-Hispanic blacks, current smokers, and middle-aged patients.

Inflammation may initiate and exacerbate PAD through a complex constellation of vascular effects. These include the adherence of leukocytes to the vessel wall; increased membrane permeability; and the production of cytokines, leading to the recruitment of macrophages and the proliferation of macrophages and smooth muscle cells within the vessel wall.^{5–9} Fibrinogen is also associated with clotting, potentially predisposing patients to thrombus formation,¹⁰ although this effect may be limited to those aged >45 years.¹¹

In the present study, inflammation exhibited a stronger association with PAD in non-Hispanic blacks than in non-Hispanic whites or Mexican Americans. Non-Hispanic blacks have been shown to have significantly higher levels of inflammatory markers than non-Hispanic whites and Hispanics.¹² After multivariate adjustment in the present study, inflammation was associated with PAD only in the third and fourth quartiles of inflammatory markers, suggesting a possible threshold effect for inflammation. Therefore, the association between inflammation and PAD may have been stronger in non-Hispanic blacks because a greater percentage may have reached threshold levels of inflammation.

Table 2

Multivariate adjusted* odds ratios (ORs) of PAD (ABI <0.9) by quartiles of CRP, fibrinogen, and leukocyte count

Variable	Age, Gender, Race and Ethnicity Adjusted			Multivariate Adjusted*		
	OR	95% CI	p Trend	OR	95% CI	p Trend
CRP (mg/dl)						
≤0.05 (reference)	1.0	—		1.0	—	
0.05–0.15	1.31	0.79–2.17		1.14	0.67–1.91	
0.15–0.33	1.66	1.20–2.30 [‡]		1.47	1.04–2.08 [‡]	
>0.33	2.72	1.83–4.03 [§]	<0.001	2.14	1.41–3.25 [§]	<0.001
Fibrinogen (mg/dl)						
≤282 (reference)	1.0	—		1.0	—	
282–333	0.95	0.48–1.89		0.85	0.41–1.76	
333–376	2.26	1.18–4.32 [†]		1.87	0.98–3.56	
>376	3.19	1.70–5.98 [§]	<0.001	2.49	1.27–4.85 [‡]	<0.001
Leukocyte count (SI)						
≤4.9 (reference)	1.0	—		1.0	—	
4.9–6.1	1.15	0.65–2.03		0.91	0.50–1.68	
6.1–7.3	1.02	0.63–1.65	<0.001	0.76	0.45–1.29	0.046
>7.3	2.66	1.42–4.99 [‡]		1.67	0.84–3.31	

* Multivariate model adjusted for age, gender, race/ethnicity, education, smoking status, diabetes, physical inactivity, total cholesterol, body mass index, and systolic blood pressure.

[†] p <0.05; [‡] p <0.01; [§] p <0.001 for comparison with reference group.

CI = confidence interval.

Table 3

Multivariate adjusted* subgroup odds ratios (ORs) of PAD (ABI <0.9) for the highest compared with the 3 lowest quartiles of CRP, fibrinogen, and leukocyte count

Subgroup	CRP			Fibrinogen			Leukocyte Count		
	OR	95% CI	p Interaction	OR	95% CI	p Interaction	OR	95% CI	p Interaction
Gender									
Female	1.58	0.94–2.65		1.83	1.16–2.88 [†]		2.01	1.17–3.44 [†]	
Male	1.85	1.18–2.90 [‡]	0.618	1.84	1.15–2.94 [†]	0.576	1.81	0.99–3.31	0.899
Race									
Non-Hispanic white	1.50	0.98–2.28		1.64	1.12–2.40 [†]		2.06	1.30–3.26 [‡]	
Non-Hispanic black	3.10	1.76–5.45 [§]	0.049	3.06	1.55–6.03 [‡]	0.118	1.51	0.76–3.01	0.099
Mexican American	1.11	0.57–2.17		1.47	0.70–3.10		3.60	1.26–10.30 [†]	
Smoking status									
Never	0.92	0.48–1.76		0.83	0.51–1.35		1.68	0.85–3.32	
Former	1.98	1.16–3.38 [†]	0.172	2.17	1.43–3.31 [§]	0.006	2.09	1.21–3.60 [‡]	0.846
Current	2.47	1.35–4.53 [‡]		3.26	1.69–6.28 [‡]		2.04	0.96–4.31	
Age									
40–54.9 yrs	5.59	1.82–17.17 [‡]		3.97	1.79–8.79 [‡]		2.05	0.97–4.30	
55–69.9 yrs	2.01	1.13–3.58 [†]	0.018	3.53	2.14–5.80 [§]	<0.001	2.81	1.50–5.25 [‡]	0.057
≥70 yrs	0.98	0.65–1.48		1.03	0.75–1.41		1.57	1.00–2.47 [†]	
Obesity									
Normal weight, BMI <25 kg/m ²	2.11	1.29–3.45 [‡]		1.61	0.82–3.16		1.33	0.75–2.37	
Overweight, BMI 25–29.9 kg/m ²	1.39	0.86–2.24	0.715	1.47	0.74–2.94	0.620	2.65	1.15–6.09 [†]	0.420
Obese, BMI ≥30 kg/m ²	1.53	0.78–3.00		2.14	1.22–3.77 [†]		1.87	1.10–3.16 [†]	
Diabetes									
Nondiabetics	1.61	1.05–2.47 [†]		1.62	1.20–2.19 [‡]		2.01	1.28–3.16 [‡]	
Diabetics	2.40	1.14–5.05 [†]	0.438	3.83	1.48–9.90 [‡]	0.086	1.88	0.91–3.86	0.712

* Multivariate model adjusted for age, gender (except in the case of gender stratification), race/ethnicity (except in the case of race/ethnic stratification), education, smoking status (except in the case of smoking stratification), diabetes (except in the case of diabetes stratification), physical inactivity, total cholesterol, BMI, and systolic blood pressure.

[†] p <0.05; [‡] p <0.01; [§] p <0.001 for comparison with reference group.

BMI = body mass index; CI = confidence interval.

The present study also found the relation between inflammation and PAD to be significantly modified by smoking status; the relation was strongest in current smokers and present, although to a weaker extent, in former smokers.

This suggests a prolonged effect of smoking on the relation between inflammation and PAD. These results are consistent with studies using other measures of subclinical atherosclerosis.^{13,14} Smoking has been associated with higher

levels of cell adhesion molecules and cytokines,^{15,16} which may underlie these results.

In the present study, the relation between inflammation and PAD was progressively weaker in older age groups. Certain data have shown that risk factors such as smoking and hypertension, which identify high-risk patients in middle-aged populations, do not demonstrate the same clinical prognosis in older patients.^{17,18} Previous studies have also shown that risk factors measured 5 to 10 years earlier are more strongly associated with the presence of disease than those concurrently measured,^{19,20} suggesting that relatively recent changes in risk factor profiles with aging can mask the effects of decades of exposure to previous risk factor levels. Results from the Honolulu Heart Study support this possibility, showing a sizable shift in risk factors between midlife and old age.²¹ Finally, the age interaction seen in the present study may also be due to the presence of high levels of subclinical and clinical disease in elderly participants and low levels in younger patients.¹⁷

Among these data, a greater leukocyte count, a routine, inexpensive clinical measure, was associated with PAD. The Framingham Offspring Study found a relation between a greater leukocyte count and the incidence of a composite outcome consisting of intermittent claudication, coronary heart disease, and cerebrovascular disease, and a case-control study showed similar findings.^{22,23} The present study data extend those findings to PAD as measured by ABI, which includes patients without symptoms such as intermittent claudication. Leukocyte count deserves consideration as an inexpensive means of assessing inflammation for the purposes of risk stratification in populations with limited health care resources.

The present study has several strengths. It provides evidence from a large, nationally representative sample of United States adults aged ≥ 40 years; multiple indexes of inflammation were measured; the associations were assessed across a range of important subgroups; and a strict protocol was used for ABI measurements that included quality control procedures.¹ However, the cross-sectional design of this study does not allow determination of the temporal relations between inflammation and PAD. Future prospective studies on the relation between inflammation and PAD or ABI, and other measures of subclinical disease, are needed to determine the causal role of inflammation in the development of PAD and to further examine the need for targeted anti-inflammatory treatment in population subgroups.

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