

# Inflammatory Markers and Cardiovascular Disease (The Health, Aging and Body Composition [Health ABC] Study)

Matteo Cesari, MD, Brenda W.J.H. Penninx, PhD, Anne B. Newman, MD, MPH, Stephen B. Kritchevsky, PhD, Barbara J. Nicklas, PhD, Kim Sutton-Tyrrell, RN, DRPH, Russell P. Tracy, PhD, Susan M. Rubin, MPH, Tamara B. Harris, MD, MS, and Marco Pahor, MD

This study investigates the association of several inflammatory markers with subclinical and clinical cardiovascular disease in older men and women. Data are from the baseline assessment of 3,045 well-functioning persons aged 70 to 79 years, participating in the Health, Aging and Body Composition study. The study sample was divided into 3 groups: "cardiovascular disease" (diagnosis of congestive heart failure, coronary artery disease, peripheral artery disease, or stroke), "subclinical cardiovascular disease" (positive findings on the Rose questionnaire for angina or claudication, ankle-brachial index <0.9, or electrocardiographic abnormalities), and "no cardiovascular disease." Serum levels of interleukin (IL)-6, C-reactive protein (CRP), tumor necrosis factor (TNF)- $\alpha$ , and the soluble receptors IL-6 soluble receptor, IL-2 soluble receptor, TNF soluble receptor I, and TNF soluble receptor II were assessed. Of those with IL-6 levels in the highest compared with the lowest tertile, the odds ratio (OR) for subclinical

cardiovascular disease was 1.58 (95% confidence interval [CI] 1.26 to 1.97) and for clinical cardiovascular disease was 2.35 (95% CI 1.79 to 3.09). A similar association was found for TNF- $\alpha$  (OR 1.48, 95% CI 1.16 to 1.88 and OR 2.05, 95% CI 1.55 to 2.72, respectively). In adjusted analyses, CRP was not significantly associated with overall subclinical or clinical cardiovascular disease, although additional analyses did find a strong specific association between CRP and congestive heart failure (OR 1.64, 95% CI 1.11 to 2.41). Of the soluble cytokine receptors, only TNF soluble receptor I showed a significant association with clinical cardiovascular disease. Thus, our findings suggest an important role for IL-6 and TNF- $\alpha$  in clinical as well as subclinical cardiovascular disease. In this study, CRP had a weaker association with cardiovascular disease than the cytokines. ©2003 by Excerpta Medica, Inc. (Am J Cardiol 2003;92:522-528)

**C**ytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)- $\alpha$ , are soluble polypeptides acting as important humoral regulators in immunoregulation, hematopoiesis, and the inflammatory cascade.<sup>1,2</sup> C-reactive protein (CRP) is an end-product of

inflammation whose synthesis by the liver is stimulated by cytokines.<sup>3</sup> Circulating cytokine receptors may provide additional information in chronic inflammatory processes because they generally have a longer half-life than the cytokines themselves and, therefore, show more constant levels over time.<sup>4,5</sup> Consequently, it has been hypothesized that the association of cytokine receptors with cardiovascular disease status may be stronger than the association with cytokines.<sup>6</sup> However, epidemiologic data on the association between inflammatory markers and cardiovascular disease is still limited, especially regarding early phases of the pathology, when the disease is not yet clinically evident. The present study examines the association between several markers of inflammation with the presence of subclinical and clinical cardiovascular disease in a large sample of older African American and white men and women.

## METHODS

**Study population:** Data are from the baseline assessment of the Health, Aging and Body Composition (Health ABC) study. This is a 7-year prospective cohort study, sponsored by the National Institute on Aging, investigating the impact of changes in body composition and health conditions on age-related

From the Sticht Center on Aging, Wake Forest University Health Sciences, Winston-Salem, North Carolina; Department of Geriatrics, Catholic University of Sacred Heart, Rome, Italy; Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania; Department of Preventive Medicine, University of Tennessee, Memphis, Tennessee; Department of Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania; Department of Biochemistry, University of Vermont, Burlington, Vermont; Department of Medicine, University of California, San Francisco, California; and the Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, Bethesda, Maryland. This study was supported by the National Institute on Aging (NIA) contract numbers N01-AG-6-2106, N01-AG-6-2102, and N01-AG-6-2103, the American Heart Association (grant number 9970066N), Dallas, Texas; and the Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Rome, Italy. Data analyses were supported by the Wake Forest University Claude D. Pepper Older Americans Independence Center, Winston-Salem, North Carolina (NIA grant 1P30-AG-21332-01). Manuscript received March 14, 2003; revised manuscript received and accepted May 16, 2003.

Address for reprints: Matteo Cesari, MD, Sticht Center on Aging, Wake Forest University Health Sciences, One Medical Center Boulevard, Winston-Salem, North Carolina 27157. E-mail: mcesari@wfubmc.edu.

physiologic and functional status. Participants, aged 70 to 79 years, were recruited from April 1997 to June 1998. They were drawn from a sample of Medicare beneficiaries residing in the areas surrounding Pittsburgh, Pennsylvania, and Memphis, Tennessee. Subjects were considered eligible if they reported no difficulty walking 1/4 mile, climbing 10 steps, or performing basic activities of daily living. Participants also had to be free of life-threatening illness and plan to remain in the geographic area for at least 3 years. A total of 3,075 subjects (50% men, 43% African Americans) were enrolled and completed baseline evaluation.

Data on inflammatory markers were missing for 30 participants, so this analysis is based on the remaining 3,045 participants. All participants signed an informed consent form that was approved by the institutional review boards of the clinical sites.

**Cardiovascular disease status:** We divided the study sample into 3 groups: participants with clinical cardiovascular disease, those with subclinical cardiovascular disease, and those with no clinical or subclinical cardiovascular disease. Participants were grouped in the clinical or subclinical cardiovascular disease groups according to the criteria diagnosed and treated versus undiagnosed and untreated disease. In this latter group, even if there was evidence of disease, the participant was unaware of symptoms or symptoms were not sufficiently severe enough to seek treatment.

**Clinical cardiovascular disease:** Based on self-reported history and the use of selected drugs, disease algorithms were created by Health ABC investigators to mirror the adjudicated diagnoses in the Cardiovascular Health Study.<sup>7</sup> Medications taken in the past 2 weeks were recorded with their brand name and a 7-digit code, which identifies specific drug products, strengths, and pharmaceutical forms.<sup>8</sup> Each ingredient in the drug product was identified with an 8-digit code, according to the Iowa Drug Information System code. This method for collecting and processing drug information has proved valid and reliable for epidemiologic purposes.<sup>9,10</sup> Clinical cardiovascular disease was considered present if  $\geq 1$  of these conditions were present: congestive heart failure (39 participants, 1.3%), coronary artery disease (including myocardial infarction [88 participants], angina [508 participants], surgical or percutaneous revascularization [60 participants]: 595 participants, 19.5%), stroke (220 participants, 7.2%), peripheral vascular disease (157 participants, 5.2%), and pacemaker (25 participants, 0.8%). Overall, 820 participants (26.9%) were identified for this group.

**Subclinical cardiovascular disease:** This group was defined as the absence of clinical cardiovascular disease but the presence of any of the following: positive results on the Rose questionnaire for angina (53 participants, 1.7%) or claudication (83 participants, 2.7%),<sup>11</sup> an ankle-brachial index  $< 0.9$  (227 participants, 7.5%),<sup>12</sup> or the presence of electrocardiographic abnormalities (1,053 participants, 34.6%).<sup>13-15</sup> Ankle-brachial index was the ratio calculated as the mean of 2 measurements of systolic blood pressure on the right

arm and both legs using an 8-MHz Doppler stethoscope. Electrocardiographic abnormalities were detected by a 12-lead electrocardiogram using a standardized protocol by technicians trained and certified by the core electrocardiographic laboratory at St. Louis University. All data were acquired using the Marquette Electronic MAC PC Resting ECG Analysis System (Marquette Electronics Inc., Milwaukee, Wisconsin). Locally, electrocardiograms were visually inspected for evidence of baseline wander, excess artifacts, and lead reversal. Clinical physicians examined all abnormal electrocardiograms. Electrocardiographic data were transmitted daily to the core electrocardiographic laboratory, where the quality of the electrocardiogram was assessed and the interpretation was confirmed. In line with previous studies on subclinical cardiovascular disease, these conditions were considered as electrocardiographic abnormalities in the present study: atrial fibrillation; Mobitz type II atrium-ventricular block; long PR interval; short PR interval; Wolf-Parkinson-White syndrome; ST- or T-wave abnormalities; left bundle branch block; right bundle branch block; intraventricular block; left anterior hemiblock; left anterior hemiblock with right bundle branch block; incomplete left bundle branch block; and left ventricular hypertrophy.<sup>13-15</sup> Overall, 1,195 participants (39.2%) qualified on  $\geq 1$  criteria for subclinical cardiovascular disease.

**No cardiovascular disease:** The remaining 1,030 participants without evidence of clinical or subclinical cardiovascular disease were considered as having no cardiovascular disease.

**Inflammatory markers:** Measures of IL-6, TNF- $\alpha$ , CRP, and soluble receptors of IL-6, IL-2, and TNF- $\alpha$  receptors I and II were obtained from frozen stored serum collected by venipuncture after an overnight fast at baseline. Soluble receptors were only assessed in a random subgroup of 499 participants. Blood samples were obtained in the morning (mean time 9:25 A.M.; 50% of the patients had blood sampling between 8:55 A.M. and 9:58 A.M.) and, after processing, the specimens were aliquoted into cryovials, frozen at  $-70^{\circ}\text{C}$ , and shipped to the Health ABC Core Laboratory at the University of Vermont. Cytokines and cytokine soluble receptors were measured in duplicate by an enzyme-linked immunosorbent assay kit from R&D Systems (Minneapolis, Minnesota). The detectable limit for IL-6 (using the HS600 Quantikine kit) was 0.10 pg/ml, 0.18 pg/ml for TNF- $\alpha$  (using the HSTA50 kit), 6.5 pg/ml for IL-6 soluble receptor (using the DR600 kit), 3 pg/ml for TNF soluble receptor I (using the DRT100 kit), 1 pg/ml for TNF soluble receptor II (using the DRT200 kit), and  $< 10$  pg/ml for IL-2 soluble receptor (using the DR2A00 kit). Serum levels of CRP were also measured in duplicate by enzyme-linked immunosorbent assay based on purified protein and polyclonal anti-CRP antibodies (Calbiochem, San Diego, California). The CRP assay was standardized according to the World Health Organization's First International Reference Standard, with a sensitivity of 0.08  $\mu\text{g/ml}$ . Blind duplicate analyses for IL-6, CRP, and TNF- $\alpha$  showed an

	No Cardiovascular Disease (n = 1,030)	Subclinical Cardiovascular Disease (n = 1,195)	p Value*	Clinical Cardiovascular Disease (n = 820)	p Value*
Age (yrs)	73.8 $\pm$ 0.1	74.2 $\pm$ 0.1	<0.001	74.5 $\pm$ 0.1	<0.001
Women	57.8%	53.4%	0.04	40.7%	<0.001
Race (white)	65.7%	52.6%	<0.001	57.8%	<0.001
Site (Memphis)	48.9%	53.0%	0.06	47.9%	0.67
Education			<0.001		0.002
Less than high school	20.2%	28.1%		27.1%	
High school graduate	34.1%	33.2%		30.2%	
Postsecondary	45.6%	38.6%		42.7%	
Smoking			0.003		<0.001
Never	49.8%	43.8%		36.1%	
Former	41.9%	44.5%		53.1%	
Current	8.3%	11.7%		10.9%	
Diabetes mellitus	10.2%	15.4%	<0.001	21.0%	<0.001
Systemic hypertension	51.6%	61.6%	<0.001	72.8%	<0.001
Chronic obstructive pulmonary disease	8.1%	9.5%	0.25	8.9%	0.52
Cancer	20.4%	16.9%	0.04	19.8%	0.74
Osteoarthritis	15.8%	17.4%	0.32	16.7%	0.61
Body mass index (kg/m <sup>2</sup> )	27.0 $\pm$ 0.1	27.7 $\pm$ 0.1	0.002	27.5 $\pm$ 0.2	0.03
Total cholesterol (mg/dl)	206.1 $\pm$ 1.2	203.6 $\pm$ 1.1	0.12	197.4 $\pm$ 1.4	<0.001
High-density lipoprotein cholesterol (mg/dl)	56.0 $\pm$ 0.5	54.8 $\pm$ 0.5	0.12	50.6 $\pm$ 0.6	<0.001
Low-density lipoprotein cholesterol (mg/dl)	123.4 $\pm$ 1.1	122.4 $\pm$ 1.0	0.51	117.9 $\pm$ 1.2	0.001
Triglycerides (mg/dl)	136.7 $\pm$ 2.4	133.9 $\pm$ 2.1	0.39	147.4 $\pm$ 3.4	0.009
Creatinine (mg/ml)	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	0.02	1.1 $\pm$ 0.1	<0.001
Albumin (mg/ml)	4.0 $\pm$ 0.1	4.0 $\pm$ 0.1	0.91	4.0 $\pm$ 0.1	0.22
Nonsteroidal anti-inflammatory drugs	43.8%	46.5%	0.20	69.3%	<0.001
Angiotensin-converting enzyme inhibitors	10.6%	13.6%	0.03	22.9%	<0.001
Statins	8.8%	9.4%	0.66	23.2%	<0.001
Corticosteroids	2.3%	1.9%	0.51	2.7%	0.63

\*p Value is based on comparison with the no cardiovascular disease group.

average interassay coefficient of variation of 10.3%, 8.0%, and 15.8%, respectively. Circulating IL-6 and CRP levels obtained from 1 time point have been shown to be reproducible and representative over extended time periods.<sup>16,17</sup>

**Covariates:** Covariates included sociodemographic variables (age, gender, race, study site, education), co-morbidity (as assessed by the adjudicated presence of diabetes mellitus, systemic hypertension, chronic obstructive pulmonary disease, cancer, and osteoarthritis), as well as physical and biologic parameters, including smoking status, body mass index (measured weight in kilograms divided for height in meters squared), total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatinine, albumin (all measured by a colorimetric technique on a Johnson & Johnson Vitros 950 analyzer, New Brunswick, New Jersey). The use of nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, statins, and systemic corticosteroids was assessed using Iowa Drug Information System codes.

**Statistical analyses:** Differences in proportions and means of covariates across cardiovascular disease groups were assessed using chi-square and analysis of variance statistics, respectively. Continuous levels and tertiles of inflammatory markers were then compared across the 3 cardiovascular disease groups, with the "no cardiovascular disease" group as the reference group. Because serum levels of inflammatory markers

were not normally distributed (except for IL-6 soluble receptor), median values with twenty-fifth to seventy-fifth percentile ranges were reported, and p values were based on Mann-Whitney U statistics. Multinomial logistic regression analyses were used to assess the association between inflammatory marker levels (log-transformed values as well as tertile groups) and the 3 cardiovascular disease groups, again with the no cardiovascular disease group as the reference group. Odds ratios (ORs) and 95% confidence intervals (CIs) were adjusted for age, gender, race, and all other covariates that had a significant univariate association with cardiovascular disease status. Similar analyses were conducted with specific subclinical and clinical diseases as the outcomes.

## RESULTS

Mean age of the study sample was 74.2 years (SD  $\pm$  2.9 years); 51.5% were women and 58.4% were white. The main population characteristics according to cardiovascular disease status are listed in Table 1. Participants with either subclinical or clinical cardiovascular disease were significantly older than those without cardiovascular disease. They were also more likely to be men or African American, to be former or current smokers, or to have diabetes or hypertension than those without cardiovascular disease. Participants with cardiovascular disease were more likely to have a higher body mass index and higher serum creatinine levels and lower total, high-density lipoprotein, and

<b>TABLE 2</b> Crude Analyses of Levels of Inflammatory Markers According to Cardiovascular Disease Status					
	No Cardiovascular Disease (n = 1,030)	Subclinical Cardiovascular Disease (n = 1,195)	p Value*	Clinical Cardiovascular Disease (n = 820)	p Value*
<b>IL-6 (pg/ml)</b>					
Median (25%–75%)	1.6 (1.1–2.4)	1.9 (1.3–2.9)	<0.001	2.1 (1.5–3.2)	<0.001
Tertile 1 (≤1.4)	42.4%	32.4%		22.9%	
Tertile 2 (1.4–2.4)	32.5%	32.3%	<0.001	36.0%	<0.001
Tertile 3 (≥2.4)	25.1%	35.3%		41.1%	
<b>CRP (μg/ml)</b>					
Median (25%–75%)	1.5 (0.9–2.9)	1.8 (1.0–3.2)	0.001	1.8 (1.0–3.4)	0.002
Tertile 1 (≤1.2)	37.3%	31.2%		31.1%	
Tertile 2 (1.2–2.5)	32.1%	34.5%	0.01	33.2%	33.2%
Tertile 3 (≥2.5)	30.6%	34.3%		35.7%	
<b>TNF-α (pg/ml)</b>					
Median (25%–75%)	3.0 (2.3–3.8)	3.2 (2.4–4.1)	0.001	3.5 (2.7–4.5)	<0.001
Tertile 1 (≤2.7)	39.6%	33.5%		25.3%	
Tertile 2 (2.7–3.7)	34.0%	33.8%	0.002	32.0%	<0.001
Tertile 3 (≥3.7)	26.4%	32.7%		42.7%	
	(n = 158)	(n = 201)		(n = 140)	
<b>IL-2 soluble receptor (mg/ml)</b>					
Median (25%–75%)	1.2 (1.0–1.6)	1.2 (0.9–1.6)	0.27	1.4 (1.0–1.7)	0.27
Tertile 1 (≤1.1)	31.2%	37.3%		30.0%	
Tertile 2 (1.1–1.5)	35.7%	33.8%	0.46	30.0%	0.42
Tertile 3 (≥1.5)	33.1%	28.9%		40.0%	
<b>IL-6 soluble receptor (mg/ml)</b>					
Median (25%–75%)	34.6 (29.6–41.7)	32.5 (27.1–38.9)	0.007	33.8 (29.1–40.3)	0.35
Tertile 1 (≤30.1)	27.2%	39.3%		31.4%	
Tertile 2 (30.1–37.7)	32.9%	32.3%	0.03	35.7%	0.45
Tertile 3 (≥37.7)	39.9%	28.4%		32.9%	
<b>TNF soluble receptor I (mg/ml)</b>					
Median (25%–75%)	1.5 (1.3–1.8)	1.5 (1.3–1.8)	0.82	1.7 (1.4–2.0)	0.001
Tertile 1 (≤1.4)	34.2%	36.0%		28.3%	
Tertile 2 (1.4–1.7)	39.2%	33.5%	0.51	26.8%	0.004
Tertile 3 (≥1.7)	26.6%	30.5%		44.9%	
<b>TNF soluble receptor II (mg/ml)</b>					
Median (25%–75%)	3.3 (2.9–3.8)	3.4 (2.8–4.0)	0.90	3.7 (3.1–4.4)	0.003
Tertile 1 (≤3.1)	34.0%	37.2%		26.9%	
Tertile 2 (3.1–3.8)	40.5%	30.7%	0.14	29.1%	0.004
Tertile 3 (≥3.8)	25.5%	32.2%		44.0%	

\*p Value based on chi-square statistics for categoric variables and Mann-Whitney U statistics for continuous variables to compare cardiovascular disease groups with the no cardiovascular disease group.

low-density lipoprotein cholesterol than subjects without clinical cardiovascular disease. Participants without cardiovascular disease less frequently used non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, and statins.

Spearman correlation analyses were performed for all cytokines and soluble receptors. A stronger correlation existed between IL-6 and CRP ( $r = 0.465$ ,  $p < 0.001$ ) than between TNF- $\alpha$  and IL-6 ( $r = 0.274$ ,  $p < 0.001$ ) or between TNF- $\alpha$  and CRP ( $r = 0.125$ ,  $p < 0.001$ ). TNF soluble receptors I and II showed a strong intercorrelation ( $r = 0.834$ ), but also with IL-2 soluble receptor ( $r = 0.637$  for TNF soluble receptor I and 0.647 for TNF soluble receptor II) and TNF- $\alpha$  ( $r = 0.573$  for TNF soluble receptor I and 0.592 for TNF soluble receptor II; all correlations,  $p < 0.001$ ). IL-6 soluble receptor showed the weakest correlations with other markers ( $r = 0.102$ ,  $p = 0.03$ ).

Unadjusted analyses showed that subjects with subclinical or clinical cardiovascular disease had significantly higher levels of IL-6, CRP, and TNF- $\alpha$  compared with subjects without cardiovascular dis-

ease (Table 2). In the subgroup of 499 participants who had levels of soluble cytokine receptors assessed, patients with cardiovascular disease had higher levels of TNF soluble receptor I and II compared with those without cardiovascular disease. Only the IL-6 soluble receptor showed an association with subclinical cardiovascular disease ( $p = 0.007$ ).

Multinomial logistic regression analyses (Table 3)—adjusted for age, gender, race, education, smoking, diabetes, hypertension, cancer, body mass index, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride (log value), creatinine, nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, and statin use—showed significantly increased ORs for clinical and subclinical cardiovascular disease across higher IL-6 tertiles or continuous levels. For example, among those with IL-6 levels in the highest compared with the lowest tertile, the OR for subclinical cardiovascular disease was 1.58 (95% CI 1.26 to 1.97) and the OR for clinical cardiovascular disease was 2.35 (95% CI 1.79 to 3.09). A similar association was

**TABLE 3** Adjusted\* Odds Ratio (OR) for Subclinical and Clinical Cardiovascular Disease Across Inflammatory Marker Levels

	OR for Subclinical Cardiovascular Disease† (95% CI)	OR for Clinical Cardiovascular Disease† (95% CI)
IL-6 (n = 3,045)		
Per log (pg/ml) increase	1.28 (1.11–1.47)	1.67 (1.41–1.97)
Tertile 1	1	1
Tertile 2	1.17 (0.94–1.44)	1.66 (1.28–2.16)
Tertile 3	1.58 (1.26–1.97)	2.35 (1.79–3.09)
CRP (n = 3,045)		
Per log (μg/ml) increase	1.08 (0.98–1.20)	1.08 (0.96–1.21)
Tertile 1	1	1
Tertile 2	1.26 (1.02–1.55)	1.28 (0.99–1.64)
Tertile 3	1.19 (0.95–1.48)	1.34 (1.03–1.65)
TNF-α (n = 3,045)		
Per log (pg/ml) increase	1.30 (1.03–1.63)	1.83 (1.39–2.41)
Tertile 1	1	1
Tertile 2	1.21 (0.97–1.50)	1.41 (1.08–1.83)
Tertile 3	1.48 (1.16–1.88)	2.05 (1.55–2.72)
IL-2 soluble receptor (n = 499)		
Per log (mg/ml) increase	1.05 (0.58–1.89)	1.30 (0.66–2.56)
Tertile 1	1	1
Tertile 2	1.00 (0.57–1.80)	0.90 (0.46–1.76)
Tertile 3	0.95 (0.52–1.75)	1.25 (0.62–2.52)
IL-6 soluble receptor (n = 499)		
Per unit (mg/ml) increase	0.79 (0.42–1.50)	0.88 (0.39–1.99)
Tertile 1	1	1
Tertile 2	0.71 (0.40–1.26)	0.78 (0.39–1.56)
Tertile 3	0.65 (0.36–1.17)	0.84 (0.43–1.65)
TNF soluble receptor I (n = 499)		
Per log (mg/ml) increase	1.20 (0.44–3.34)	4.36 (1.35–14.14)
Tertile 1	1	1
Tertile 2	0.93 (0.54–1.61)	0.77 (0.39–1.52)
Tertile 3	1.26 (0.67–2.39)	1.99 (0.98–4.04)
TNF soluble receptor II (n = 499)		
Per log (mg/ml) increase	0.64 (0.23–1.77)	1.46 (0.51–4.22)
Tertile 1	1	1
Tertile 2	0.74 (0.42–1.29)	0.70 (0.35–1.39)
Tertile 3	1.15 (0.60–2.20)	1.54 (0.72–3.30)

\*Adjusted for age, gender, race, education, smoking, diabetes, hypertension, cancer, body mass index, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides (log value), creatinine, nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, and statin use.

†The reference group was the no cardiovascular disease group.

found for TNF-α patients in the highest tertile. They were more likely to have subclinical and clinical cardiovascular disease. TNF soluble receptor I showed a significant level of association with clinical cardiovascular disease. No other significant associations were found for cardiovascular disease status and levels of CRP, IL-2 or IL-6 soluble receptor, or TNF soluble receptor II. We explored which potential confounders were able to partly account for the association between CRP and cardiovascular disease. It appeared that when we entered hypertension, creatinine, and diabetes (the strongest confounders) into the model, the association between CRP and clinical cardiovascular disease was no longer significant (OR 1.11, 95% CI 0.99 to 1.24).

We examined whether the ratio between IL-6 and IL-6 soluble receptor had a stronger association with cardiovascular disease status than IL-6 levels alone. However, the multinomial logistic regression model that included the ratio term did not differ from the model that

included IL-6 level (chi-square values for models were 134.0 vs 137.2), indicating that the ratio term between IL-6 and IL-6 soluble receptor did not add more predictive value compared with IL-6 levels alone. Similar findings were found for the ratio terms between TNF-α and its soluble receptors. We analyzed interaction terms by race and gender groups by examining whether associations between IL-6, TNF-α, and CRP with cardiovascular disease status were similar across race and gender groups; we did not find any consistent gender or race interactions.

Finally, to evaluate whether specific subclinical and clinical cardiovascular diseases showed different patterns in inflammatory marker associations, we conducted analyses with specific subclinical and clinical cardiovascular diseases (Table 4). Results of overall outcomes were rather similar to our previous findings; however, specific high associations were found between inflammatory marker levels and congestive heart failure.

## DISCUSSION

This is the first large study in which the association between several inflammatory markers and cardiovascular disease was evaluated and in which subclinical cardiovascular disease was considered separately. Our results showed a strong independent association between inflammatory markers and the presence of cardiovascular disease. Immuno-regulating cytokines, especially IL-6, could represent important risk factors for cardiovascular disease. A link with IL-6 and TNF-α levels was also found for subclinical cardiovascular disease. Significant associations with CRP were found for only congestive heart failure and coronary heart disease, but not for subclinical cardiovascular disease. In a subgroup of 499 patients, we found an association between TNF soluble receptor I and clinical cardiovascular disease.

In the multivariate analyses, CRP had, overall, a weaker association with clinical and subclinical cardiovascular disease than IL-6 and TNF-α in our study. Several studies suggest a CRP diagnostic value for myocardial infarction, coronary artery disease, and stroke, even in apparently healthy persons.<sup>15,18–21</sup> The fact that associations between some cardiovascular diseases and CRP were weaker in our study may be due to adjustment for confounders that some previous studies did not use, such as race, education, creatinine, cancer, and angiotensin-converting enzyme inhibitor use.<sup>15,18,20–23</sup> Moreover, compared with previous studies conducted in community-dwelling

TABLE 4 Adjusted* OR (95% CI) for Specific Subclinical and Clinical Cardiovascular Diseases Across Levels of IL-6, CRP and TNF- $\alpha$ Stratified for Specific Diseases and Cardiovascular Test Results	Subclinical Cardiovascular Disease			Clinical Cardiovascular Disease			
	Electrocardiographic Abnormalities (n = 1,053)	Angina Symptoms† (n = 53)	Claudication Symptoms† (n = 83)	Ankle-Brachial Index <0.9 (n = 227)	Congestive Heart Failure (n = 39)	Coronary Artery Disease + Acute Myocardial Infarction (n = 595)	Stroke (n = 220)
IL-6							
Per log (pg/ml) increase	1.31 (1.13–1.52)	1.46 (0.92–2.33)	1.37 (0.94–2.01)	1.53 (1.19–1.97)	3.65 (2.03–6.58)	1.88 (1.52–2.31)	1.60 (1.23–2.09)
CRP							
Per log ( $\mu$ g/ml) increase	1.12 (0.99–1.26)	0.84 (0.58–1.23)	1.09 (0.83–1.45)	1.19 (0.98–1.44)	1.64 (1.11–2.41)	1.27 (1.09–1.47)	1.20 (0.98–1.45)
TNF- $\alpha$							
Per log (pg/ml) increase	1.37 (1.07–1.74)	1.53 (0.73–3.20)	3.29 (1.77–6.10)	1.47 (0.96–2.24)	3.45 (1.35–8.84)	2.26 (1.64–3.13)	1.75 (1.14–2.70)

\*Adjusted for age, gender, race, education, smoking, diabetes, hypertension, cancer, body mass index, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides (log value), creatinine, nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, and statin use. The reference group was the no cardiovascular disease group.

†As detected by the Rose questionnaire.

healthy persons, our study sample included older patients,<sup>4,18,19,22,24,25</sup> in whom the CRP and cardiovascular disease association may be weaker.<sup>18,26</sup> Overall, levels of inflammatory markers (IL-6, CRP, TNF- $\alpha$ ) in previous studies conducted in younger patients<sup>24</sup> were generally lower than the levels in our sample.

Our study has limitations. The cross-sectional study design did not allow us to directly assess an etiologic association between increased cytokine levels and cardiovascular disease, either in its subclinical or clinical form. It would be interesting to evaluate whether the presence of higher levels of IL-6 and TNF- $\alpha$  predicts future cardiovascular events, especially in patients with subclinical cardiovascular disease. Volpato et al<sup>27</sup> have already identified a relation between IL-6 levels and clinical outcomes in patients with cardiovascular disease. Another limitation is that soluble cytokine receptors were only available in a subcohort of 499 persons. Consequently, the power to detect significant associations between soluble cytokine receptor levels and cardiovascular disease status may have been too small. Because unadjusted analyses did show significant associations between higher levels of TNF soluble receptor I and II and clinical cardiovascular disease, these links deserve further investigation in a larger study.

1. Van Stick J. Interleukin-6: an overview. *Annu Rev Immunol* 1990;8:253–278.
2. Beutler M, Milsark IW, Cerami A. Cachetin/tumor necrosis factor: production, distribution, and metabolic fate in vivo. *J Immunol* 1985;135:3972–3977.
3. Mendall MA, Patel P, Asante M, Ballam L, Morris J, Strachan DP, Camm AJ, Northfield TC. Relation of serum cytokine concentrations to cardiovascular risk factors and coronary heart disease. *Heart* 1997;78:273–277.
4. Elkind MS, Cheng J, Boden-Albala B, Rundek T, Thomas J, Chen H, Rabbani LE, Sacco RL. Tumor necrosis factor receptor levels are associated with carotid atherosclerosis. *Stroke* 2002;33:31–38.
5. Dibbs Z, Thornby J, White BG, Mann DL. Natural variability of circulating levels of cytokines and cytokine receptors in patients with heart failure: implications for clinical trials. *J Am Coll Cardiol* 1999;33:1935–1942.
6. Porsch-Oezcuemez M, Kunz D, Kloer HU, Luley C. Evaluation of serum levels of solubilized adhesion molecules and cytokine receptors in coronary heart disease. *J Am Coll Cardiol* 1999;34:1995–2001.
7. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin J, Kuller LH, Manolio TA, Newman AB, O'Leary DH. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol* 1991;1:263–276.
8. Pahor M, Chrischilles EA, Guralnik J, Brown SL, Wallace RB, Carbonin P. Drug data coding and analysis in epidemiologic studies. *Eur J Epidemiol* 1994;10:405–411.
9. Di Bari M, Salti F, Nardi M, Pahor M, De Fusco C, Tonon E, Ungar A, Pini R, Masotti G, Marchionni N. Undertreatment of hypertension in community-dwelling older adults: a drug-utilization study in Dicomano, Italy. *J Hypertens* 1999;17:1633–1640.
10. Pahor M, Salive ME, Brown SL. Medication use. In: Guralnik J, Fried LP, Simonsick EM, eds. *The Women's Health and Aging Study. Health and Social Characteristics of Older Women With Disability*. NIH publication No. 95–4009 ed. Bethesda, MD: National Institutes of Health, National Institute on Aging, 1995.
11. Rose GA, Blackburn H, Gillium RF, Prineas RJ. *Cardiovascular Survey Methods*. 2nd Ed. Geneva, Switzerland: WHO, 1982:162–165.
12. Olin JW. The clinical evaluation and office based detection of peripheral arterial disease. In: Hirsch AT, Olin FW, eds. *An Office-Based Approach to the Diagnosis and Treatment of Peripheral Arterial Disease*. American Journal of Medicine Continuing Education Series. Belle Meade, NJ: Excerpta Medica Inc. 1998:10–17.
13. Kuller LH, Shemanski L, Psaty BM, Borhani NO, Gardin J, Haan MN, O'Leary DH, Savage PJ, Tell GS, Tracy RP. Subclinical disease as an independent risk factor for cardiovascular disease. *Circulation* 1995;92:720–726.
14. Newman AB, Naydeck B, Sutton-Tyrrell K, Edmundowicz D, Gottdiener J, Kuller LH. Coronary artery calcification in older adults with minimal clinical or subclinical cardiovascular disease. *J Am Geriatr Soc* 2000;48:256–263.
15. Tracy RP, Lemaitre RN, Psaty BM, Ives DG, Evans RW, Cushman M, Meilahn EN, Kuller LH. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly. *Arterioscler Thromb Vasc Biol* 1997;17:1121–1127.

16. Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological implications. *Clin Chem* 1997;43:52–58.
17. Rao KM, Pieper CS, Currie MS, Cohen HJ. Variability of plasma IL-6 and crosslinked fibrin dimers over time in community dwelling elderly subjects. *Am J Clin Pathol* 1994;102:802–805.
18. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000;342:836–843.
19. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;336:973–979.
20. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation* 1998;97:2007–2011.
21. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation* 1998;98:731–733.
22. Ford ES, Giles WH. Serum C-reactive protein and self-reported stroke: findings from the Third National Health and Nutrition Examination Survey. *Arterioscler Thromb Vasc Biol* 2000;20:1052–1056.
23. Biasucci LM, Liuzzo G, Grillo RL, Caligiuri G, Rebuzzi AG, Buffon A, Summaria F, Ginnetti F, Fadda G, Maseri A. Elevated levels of C-reactive protein at discharge in patients with unstable angina predict recurrent instability. *Circulation* 1999;99:855–860.
24. Skoog T, Dichtl W, Boquist S, Skoglund-Andersson C, Karpe F, Tang R, Bond MG, De Faire U, Nilsson J, Eriksson P, Hamstein A. Plasma tumor necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men. *Eur Heart J* 2002;23:376–383.
25. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 2000;101:1767–1772.
26. Ross T. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;362:801–809.
27. Volpato S, Guralnik J, Ferrucci L, Balfour J, Chaves P, Fried LP, Harris TB. Cardiovascular disease, interleukin-6, and risk of mortality in older women — The Women's Health and Aging Study. *Circulation* 2001;103:947–953.