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Background—Inflammation plays an important role in cardiovascular disease. The aim of this study is to investigate the predictive value of several inflammatory markers on the incidence of cardiovascular events in well-functioning older persons.

Methods and Results—The subjects were 2225 participants 70 to 79 years old, without baseline cardiovascular disease, who were enrolled in the Health, Aging, and Body Composition study. Incident coronary heart disease (CHD), stroke, and congestive heart failure (CHF) events were detected during an average follow-up of 3.6 years. Blood levels of interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor-α (TNF-α) were assessed. After adjustment for potential confounders, IL-6 was significantly associated with all outcomes (CHD events, per IL-6 SD increase: RR, 1.27; 95% CI, 1.10 to 1.48; stroke events, per IL-6 SD increase: RR, 1.45; 95% CI, 1.12 to 1.86; CHF events, per IL-6 SD increase: RR, 1.72; 95% CI, 1.40 to 2.12). TNF-α showed significant associations with CHD (per TNF-α SD increase: RR, 1.22; 95% CI, 1.04 to 1.43) and CHF (per TNF-α SD increase: RR, 1.59; 95% CI, 1.30 to 1.95) events. CRP was significantly associated with CHF events (per CRP SD increase: RR, 1.48; 95% CI, 1.23 to 1.78). A composite summary indicator of inflammation showed a strong association with incident cardiovascular events, with an especially high risk if all 3 inflammatory markers were in the highest tertile.

Conclusions—Findings suggest that inflammatory markers are independent predictors of cardiovascular events in older persons. **(*Circulation*. 2003;108:2317-2322.)**

Key Words: cytokines ■ cardiovascular disease ■ inflammation

Many cardiovascular events occur in persons without documented clinical or subclinical cardiovascular disease or known cardiovascular risk factors.[1] Furthermore, the predictive validity of traditional cardiovascular risk factors diminishes with increasing age.[2,3] Consequently, to better identify patients at high risk for cardiovascular events, new predictive markers need to be established, especially in aged populations.

Various reports have indicated that inflammatory markers appear to be predictive of cardiovascular events,[4–11] but most of these have focused on relatively young populations.[5,7–10] Moreover, few articles have explored the predictive value of inflammatory markers on cardiovascular events in cardiovascular disease–free subjects,[4,5,7,9,10,12,13] and even fewer have considered multiple markers, such as interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor-α (TNF-α), simultaneously.[4,14] In fact, most studies have focused only on CRP.[9–12,15–18] A recent longitudinal study among elderly subjects without previous myocardial infarction has shown a stronger role for IL-6 in the prediction of congestive heart failure (CHF) than for CRP or TNF-α.[4]

The aim of the present study was to assess the incidence of coronary heart disease (CHD), stroke, and CHF events according to serum levels of inflammatory markers (CRP, IL-6, and TNF-α) in a large sample of older, well-functioning subjects.

Methods

Data are from the Health, Aging, and Body Composition (Health ABC) study, a 7-year prospective cohort study designed to investigate the impact of changes in body composition and health conditions on age-related physiological and functional status. Participants, 70 to 79 years of age, were recruited between April 1997 and June 1998 from a list of Medicare beneficiaries residing in the areas surrounding Pittsburgh, Pa, and Memphis, Tenn. Eligibility criteria

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included (1) no difficulty walking one quarter mile, climbing 10 steps, or performing basic activities of daily living; (2) no life-threatening illness; and (3) no plans to leave the area for 3 years. In all, 3075 persons were enrolled and completed baseline evaluation. Participant had telephone contacts every 6 months and clinical visits every year during which health status was assessed and data about interim hospitalizations or major outpatient procedures were collected. The presence of clinical disease at baseline was ascertained by use of algorithms, mirroring adjudicated diagnoses in the Cardiovascular Health Study.[19]

The present study is based on 2225 participants; we excluded 34 participants because of missing data on all inflammatory markers and 816 participants with ≥1 of the following cardiovascular diseases at baseline: CHF, CHD, stroke, or peripheral vascular disease, or having a pacemaker. All participants gave informed consent; the protocol was approved by the Institutional Review Boards of the clinical sites.

Inflammatory Markers

Measures of levels of IL-6, TNF-α, and CRP were obtained from frozen stored serum (IL-6) or plasma (TNF-α) collected by venipuncture after an overnight fast at baseline. Blood samples were obtained in the morning, and after processing, the specimens were divided into aliquots in cryovials, frozen at −70°C, and shipped to the Health ABC Core Laboratory at the University of Vermont. Cytokines were measured in duplicate by ELISA kit from R&D Systems. The detectable limit for IL-6 (by HS600 Quantikine kit) was 0.10 pg/mL and for TNF-α (by HSTA50 kit) 0.18 pg/mL. Serum levels of CRP were also measured in duplicate by ELISA on the basis of purified protein and polyclonal anti-CRP antibodies (Calbiochem). The CRP assay was standardized according to World Health Organization First International Reference Standard with a sensitivity of 0.08 mg/L. The lower detection limit for CRP was 0.007 mg/L. Blind duplicate analyses (n=150) for IL-6, CRP, and TNF-α showed interassay coefficients of variation of 10.3%, 8.0%, and 15.8%, respectively.

Cardiovascular Events

Outcomes were as follows: incident CHD, defined by coronary death or any overnight hospitalization in an acute care hospital for acute myocardial infarction or angina; incident stroke, defined as fatal and nonfatal stroke events; and incident CHF, defined as any overnight hospitalization in an acute care hospital for CHF during the follow-up.

Participants were questioned about any hospitalizations for CHD, stroke, or CHF every 6 months. When an event was reported, hospital records were collected and verified by a Health ABC Disease Adjudicator at each site. Date and causes of death were taken from the death certificate. Follow-up time was defined by the time from the baseline visit until the first event date (for those who had an event) or was censored at the last contact date (for those who did not have any event or were lost to follow-up) or the day of death (for those who died of noncardiovascular causes).

Covariates

Covariates included sociodemographic variables (age, gender, race, study site, education), comorbidity (adjudicated presence of diabetes, hypertension, chronic obstructive pulmonary disease [COPD], and cancer), and physical and biological parameters, including smoking status, body mass index (BMI, weight in kilograms divided by height in meters squared), total cholesterol, HDL cholesterol, triglycerides, creatinine, and albumin (all measured by a colorimetric technique on a Johnson & Johnson Vitros 950 analyzer). LDL levels were calculated by use of the Friedewald equation. Medications taken in the previous 2 weeks were brought in, recorded, and coded according to the Iowa Drug Information System. By use of this system, nonsteroidal antiinflammatory drugs, systemic corticosteroids, angiotensin-converting enzyme inhibitors, and statins were identified.

Statistical Analyses

Differences in proportions and means of covariates across persons with and without incident cardiovascular events during follow-up were assessed by use of χ^2 and ANOVA statistics, respectively. Because levels of inflammatory markers were not normally distributed, median values with interquartile ranges were reported, and probability values were based on Mann-Whitney U statistics. To assess the relative risk for incident cardiovascular events, Cox proportional hazards analyses were performed; log-transformed values for inflammatory markers and tertile groups were used for these analyses. To permit direct comparison between inflammatory markers, risks are shown per SD in log(inflammatory marker) increase. Relative risks (RRs) and 95% CIs were adjusted for age, gender, race, and all other covariates with a significant univariate association with onset of cardiovascular events. Separate analyses were conducted to evaluate relative risks for CHD, stroke, and CHF events.

Separate Cox proportional hazard analyses, adjusted for age, gender, and race, were performed for inflammation and other established cardiovascular risk factors (IL-6, CRP, and TNF-α in their highest tertile separately or together, current smoking, total cholesterol >240 mg/dL, LDL cholesterol ≥130 mg/dL, HDL cholesterol ≤40 mg/dL, hypertension, diabetes, obesity). Risks for events were also calculated according to a composite summary indicator of inflammation as the number of inflammatory markers in the highest tertile.

Results

The 2225 participants (992 men [44.6%] and 1233 women [55.4%]) had a mean age of 74.0 years (SD, 2.8 years) and were more likely to be white (58.7%). The average follow-up time was 3.6 years (SD, 0.9 years). During this period, 188 CHD events, 60 stroke events, and 92 CHF events were reported. The incidence of cardiovascular events was significantly higher for those who smoked or had diabetes, hypertension, COPD, high BMI, high levels of triglyceride and creatinine, and low levels of HDL cholesterol and albumin (Table 1).

CRP and IL-6 levels were correlated most strongly (Spearman $r=0.475$, $P<0.001$), but significant correlations were also present between TNF-α and IL-6 ($r=0.269$, $P<0.001$) and between TNF-α and CRP ($r=0.132$, $P<0.001$).

In univariate analyses (Table 2), participants with incident CHD, stroke, and CHF events had higher levels of IL-6 at baseline. TNF-α levels were higher in those with CHD and CHF events, whereas high CRP levels were found only for those with CHF events.

Table 3 reports the predictive effect of levels of inflammatory markers on cardiovascular events. The incidence of CHD events was predicted by high levels of IL-6 (RR, 1.27; 95% CI, 1.10 to 1.48) and TNF-α (RR, 1.22; 95% CI, 1.04 to 1.43). IL-6 level was the only inflammatory marker that significantly predicted stroke events (RR, 1.45; 95% CI, 1.12 to 1.86). The incidence of CHF events was predicted by high levels of IL-6 (RR, 1.72; 95% CI, 1.40 to 2.12), CRP (RR, 1.48; 95% CI, 1.23 to 1.78), and TNF-α (RR, 1.59; 95% CI, 1.30 to 1.95). No significant gender or race interaction was found in the adjusted analyses for cardiovascular events. Because albumin is considered a negative acute-phase reactant, we reran analyses excluding it as a covariate to avoid an overadjustment of our models, but results were very similar.

Separate proportional hazard models were fit for incident CHD, stroke, and CHF events to compare inflammatory markers and established cardiovascular risk factors (Table 4).

TABLE 1. Participants' Characteristics

	No Cardiovascular Events, mean±SD or % (n=1950)	Cardiovascular Events, mean±SD or % (n=275)	*P*
Demographics			
Age, y	74.0±2.8	74.3±3.0	0.12
Female gender	56.9	45.1	<0.001
White race	58.5	60.4	0.55
Site, Memphis	51.1	50.9	0.95
Education			0.11
Less than high school	23.8	29.4	
High school	33.8	32.7	
Postsecondary	42.4	37.9	
Smoking			<0.001
Never	48.2	35.4	
Former	42.0	52.2	
Current	9.8	12.4	
Baseline comorbid conditions			
Diabetes	11.9	20.4	<0.001
Hypertension	55.9	64.0	0.01
COPD	8.5	10.9	0.19
Cancer	18.9	16.0	0.25
Objective tests			
BMI, kg/m^2	27.3±4.9	28.0±5.1	0.03
Total cholesterol, mg/dL	205.0±37.8	203.4±37.5	0.52
HDL cholesterol, mg/dL	55.8±16.9	52.0±18.7	0.001
LDL cholesterol, mg/dL	122.9±34.5	122.7±34.5	0.92
Triglycerides, mg/dL*	116.0 (87.0–159.0)	123.0 (90.0–165.0)	0.04
Creatinine, mg/mL	1.0±0.4	1.1±0.4	0.07
Albumin, mg/mL	39.8±3.1	39.1±3.2	<0.001
Medication use			
Nonsteroidal antiinflammatory drugs	22.6	22.9	0.91
Angiotensin-converting enzyme inhibitors	12.2	12.4	0.94
Corticosteroids	2.0	2.9	0.33
Statins	9.0	10.2	0.52

*Expressed as median (25%–75%).

For each outcome, inflammatory markers, especially IL-6, were as potent or more potent predictors of disease onset compared with traditional risk factors. Elevation of all 3 markers was the strongest predictor of both CHD and CHF risk.

Restricted analyses in participants without traditional risk factors (hypertension, diabetes, BMI >30 kg/m^2, total cholesterol >240 mg/dL, HDL cholesterol ≤40 mg/dL, and LDL cholesterol ≥130 mg/dL) were performed to identify the predictive value of inflammatory markers among persons with a low cardiovascular risk profile. In this restricted sample (17 events/307 participants; 5.5%), inflammatory markers were still able to predict cardiovascular events (IL-6: RR, 1.68; 95% CI, 1.11 to 2.55; CRP: RR, 1.34; 95% CI, 0.86

TABLE 2. Inflammatory Markers Levels (Median, Interquartile Range) According to Incident CHD, Stroke, and CHF Events

	No Events (n=1950)	CHD Events (n=188)	*P**	Stroke Events (n=60)	*P**	CHF Events (n=92)	*P**
IL-6, pg/mL	1.68 (1.16–2.54)	2.11 (1.40–3.13)	<0.001	2.41 (1.78–3.17)	<0.001	2.62 (1.62–4.35)	<0.001
CRP, mg/L	1.61 (0.98–3.01)	1.74 (1.04–3.10)	0.193	1.99 (1.04–3.60)	0.102	2.70 (1.47–4.41)	<0.001
TNF-α, pg/mL	3.02 (2.35–3.86)	3.50 (2.67–4.66)	<0.001	3.20 (2.38–4.42)	0.212	3.40 (2.67–5.33)	<0.001

**P* values vs No Events are based on Mann-Whitney *U* statistics.

TABLE 3. Adjusted* RR (95% CI) for New CHD, Stroke, and CHF Events According to Levels of Inflammatory Markers

	CHD Events (n=188)	Stroke Events (n=60)	CHF Events (n=92)
IL-6, pg/mL			
Per log(IL-6) increase	1.46 (1.16–1.84)	1.78 (1.20–2.65)	2.34 (1.70–3.24)
Per SD† in log(IL-6) increase	1.27 (1.10–1.48)	1.45 (1.12–1.86)	1.72 (1.40–2.12)
Tertile I‡: 0.21–1.34 (1.02)	1	1	1
Tertile II‡: 1.35–2.29 (1.73)	1.21 (0.81–1.80)	2.01 (0.86–4.67)	2.22 (1.02–4.84)
Tertile III‡: 2.30–15.96 (3.24)	1.61 (1.09–2.38)	3.70 (1.67–8.21)	5.08 (2.45–10.53)
CRP, mg/L			
Per log(CRP) increase	1.13 (0.95–1.35)	1.22 (0.90–1.67)	1.60 (1.28–2.00)
Per SD† in log(CRP) increase	1.11 (0.96–1.29)	1.18 (0.91–1.53)	1.48 (1.23–1.78)
Tertile I‡: 0.15–1.15 (0.85)	1	1	1
Tertile II‡: 1.16–2.50 (1.65)	1.09 (0.76–1.57)	1.04 (0.53–2.03)	1.43 (0.77–2.65)
Tertile III‡: 2.51–85.18 (3.95)	1.20 (0.83–1.75)	1.41 (0.73–2.71)	2.60 (1.45–4.67)
TNF-α, pg/mL			
Per log(TNF-α) increase	1.62 (1.10–2.38)	0.88 (0.44–1.76)	3.08 (1.88–5.05)
Per SD† in log(TNF-α) increase	1.22 (1.04–1.43)	0.95 (0.71–1.26)	1.59 (1.30–1.95)
Tertile I‡: 0.57–2.61 (2.07)	1	1	1
Tertile II‡: 2.62–3.61 (3.07)	1.45 (0.96–2.20)	0.99 (0.51–1.91)	1.67 (0.92–3.04)
Tertile III‡: 3.61–22.43 (4.42)	1.79 (1.18–2.71)	0.83 (0.41–1.66)	2.21 (1.24–3.96)

*Adjusted for age, gender, race, smoking, diabetes, hypertension, BMI, HDL cholesterol, triglyceride, and albumin.

†SD for log(IL-6)=0.64; SD for log(CRP)=0.83; SD for log(TNF-α)=0.41.

‡Lower boundary–upper boundary (median).

to 2.10; TNF-α: RR, 1.36; 95% CI, 0.86 to 2.16), and the relative risks were even somewhat higher then those found among the 1918 participants with ≥1 cardiovascular risk factors (258 events [13.5%]; IL-6: RR, 1.34; 95% CI, 1.18 to 1.52; CRP: RR, 1.20; 95% CI, 1.07 to 1.36; TNF-α: RR, 1.22; 95% CI, 1.07 to 1.40).

Finally, we conducted analyses to examine the cardiovascular risks according to a composite measure counting the number of inflammatory markers in the highest tertile (Figure 1). The adjusted relative risk for cardiovascular events was the highest when participants had the highest tertile level for all 3 inflammatory markers. Participants with all 3 markers in their highest tertile had a >2- to 3-fold increased risk for CHD (1 marker: RR, 1.17; 95% CI, 0.79 to 1.73; 2 markers: RR, 1.22; 95% CI, 0.79 to 1.88; 3 markers: RR, 2.13; 95% CI, 1.27 to 3.55) and CHF (1 marker: RR, 1.08; 95% CI, 0.56 to 2.08; 2 markers: RR, 1.97; 95% CI, 1.05 to 3.70; 3 markers: RR, 4.85; 95% CI, 2.46 to 9.52) events compared with the unexposed group. Similar but nonsignificant trends were found for stroke events.

Discussion

The present study examined the predictive value of IL-6, CRP, and TNF-α for the incidence of CHD, stroke, and CHF events in older adults without evidence of cardiovascular disease at baseline. Our findings show that all 3 inflammatory markers predicted the onset of cardiovascular events, and individuals with high levels of all 3 markers had the greatest risk of cardiovascular events. In our study, increased IL-6 level was the strongest and most consistent risk factor for cardiovascular events.

In our study, IL-6 and TNF-α showed more consistent results than CRP in predicting cardiovascular events. Recently, Vasan et al[4] explored the predictive value of these inflammatory markers on the onset of CHF in older subjects without previous myocardial infarction. Their findings are consistent with ours, showing a strong predictive value for the all markers, particularly for IL-6. Similar findings were also reported by Koukkunen et al,[14] exploring the prognostic value of these 3 inflammatory markers in older patients with CHD. Perhaps, especially because of its wide range of actions, including effects on platelets, endothelium, and factors of metabolism and coagulation, IL-6 may have a more important role in the development of CHD than some other inflammatory markers.[20]

The association between inflammatory markers and stroke has not been well studied. An inflammatory reaction is a common response of the brain parenchyma to various forms of insult. Cytokines play an important role during cerebral ischemia[21] and are involved in carotid atherosclerosis.[12] Higher CRP levels have been shown to predict stroke.[22] In our study, we found an increased risk for stroke events in participants with increased IL-6 levels but not for the other inflammatory markers.

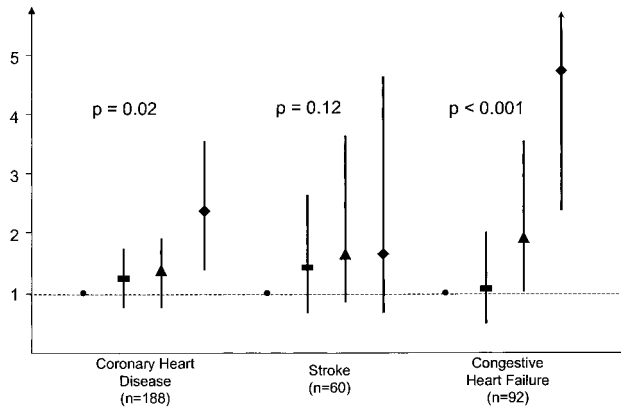
Our results confirm the strong relationship between inflammation and CHF reported in previous studies. TNF-α and

TABLE 4. Adjusted* RR (95% CI) for New CHD, Stroke, and CHF Events According to Cardiovascular Risk Factors

	CHD Events (n=188)	Stroke Events (n=60)	CHF Events (n=92)
IL-6 in highest tertile†	1.71 (1.27–2.29)	2.86 (1.71–4.80)	3.70 (2.39–5.75)
CRP in highest tertile†	1.33 (0.98–1.80)	1.64 (0.97–2.77)	2.40 (1.58–3.66)
TNF-α in highest tertile†	1.67 (1.23–2.26)	1.18 (0.69–2.03)	1.90 (1.24–2.90)
Three inflammatory markers in highest tertile†	2.29 (1.49–3.53)	1.90 (0.86–4.21)	4.20 (2.53–6.97)
Current smoking	1.32 (0.98–1.77)	1.67 (0.98–2.82)	1.18 (0.77–1.81)
Total cholesterol >240 mg/dL	1.13 (0.76–1.70)	1.21 (0.62–2.36)	0.93 (0.52–1.66)
LDL cholesterol ≥130 mg/dL	1.07 (0.80–1.44)	1.32 (0.78–2.22)	0.72 (0.47–1.12)
HDL cholesterol ≤40 mg/dL	1.19 (0.84–1.70)	2.19 (1.20–3.99)	1.74 (1.05–2.90)
Hypertension	1.28 (0.95–1.22)	1.47 (0.86–2.51)	2.18 (1.36–3.49)
Diabetes	1.90 (1.33–2.70)	2.17 (1.18–4.01)	1.81 (1.08–3.02)
BMI >30	1.51 (1.10–2.07)	1.05 (0.58–1.91)	1.68 (1.08–2.61)
IL-6‡§	1.36 (1.15–1.61)	1.53 (1.16–2.02)	1.52 (1.20–1.92)
CRP‡§	1.01 (0.85–1.20)	1.08 (0.80–1.44)	1.26 (0.99–1.59)
TNF-α‡§	1.19 (1.02–1.39)	1.01 (0.77–1.33)	1.32 (1.07–1.63)

*Adjusted for age, gender, and race in separate proportional hazard models for each cardiovascular risk factor.
†Versus rest of the participants.
‡Per SD in log(unit) increase. SD for log(IL-6)=0.64; SD for log(CRP)=0.83; SD for log(TNF-α)=0.41.
§All 3 inflammatory markers are entered simultaneously in the proportional hazard model.

IL-6 levels are associated with the severity of left ventricular dysfunction and with the degree of activation of the sympathetic and renin-angiotensin systems.[23] It has also been reported that proinflammatory cytokines might depress myocardial contractility.[24] Over the past years, a series of multicenter clinical trials using "targeted" approaches to neutralize cytokines in patients with CHF have recently been conducted.[25] Some of these trials failed to show substantial beneficial effects of these new therapies for CHF.[26] These results raise important questions about the role that cytokines play in the pathogenesis of CHF. Are higher levels of inflammatory markers part of the pathophysiological pathway leading to cardiovascular disease or just an indirect measure of subclinical disease? If the latter is shown to be true, a treatment aimed to lower inflammation might be useless.



Adjusted relative risks for new CHD, stroke, and CHF events according to number of inflammatory markers (IL-6, CRP, and TNF-α) in the highest tertiles (adjusted for age, gender, race, smoking, diabetes, hypertension, COPD, BMI, HDL cholesterol, triglyceride, creatinine, and albumin). n=2225 participants. ●, No cytokines highest tertiles (reference group); ■, highest tertile of 1 marker; ▲, highest tertiles of 2 markers; ◆, highest tertiles of 3 markers. Symbols indicate RR; lines indicate 95% CI. *P* for trend.

Our results suggest that IL-6 and TNF-α levels may be stronger predictors for incident cardiovascular events than CRP level. However, compared with most previous studies, our sample included much older persons, in whom the relationship between CRP and onset of cardiovascular events may be weaker.[27]

We also provided a comparison between cardiovascular risk factors, and high levels of inflammatory markers showed the highest associations with incident cardiovascular event. Some traditional cardiovascular risk factors failed to show a significant relationship, confirming that the aging process may diminish their reliability in the prediction of cardiovascular events.[5] In line with previous findings,[7] our restricted analyses on participants free from traditional cardiovascular risk factors showed that IL-6 level was still able to predict the new onset of cardiovascular disease. Cytokines may be involved in the destabilization and disruption of the atherosclerotic plaque. In fact, the presence of inflammation characterizes the site of plaque rupture,[28] and proinflammatory cytokines upregulate the expression of matrix metalloproteinases, which are known to be involved in the vascular remodeling and plaque disruption.[29]

The single blood sampling for cytokine dosage could be influenced by the circadian rhythm they have and could be a limitation of our study. This would tend to weaken the observed associations because of added variability.

The recent AHA/CDC Scientific Statement[30] about inflammatory markers and cardiovascular disease states that laboratory tests assessing inflammation should be limited to those that can be used in clinical settings, that have commercially

available and standardized assays, and that present adequate precision. It was suggested to limit current assays of inflammatory markers to CRP, using it as an adjunct to the major traditional risk factors. Although our study finds a better predictive value for IL-6 on the onset of cardiovascular events, we agree that CRP still maintains its importance as a marker of disease and, especially for its economic characteristics, it is more suitable than others to be used right now. However, this might change over time, when the assessment of other cytokines will become more easily available and cheaper and if our findings are confirmed by other studies.

Implementation of the assessment of inflammatory markers as additional risk factors to consider in addition to the traditional ones in the evaluation of cardiovascular event risk should be encouraged, especially in the elderly. Further studies are needed to find out whether inflammatory markers might represent valid targets for new medications able to modify the atherosclerosis process.[9,31]

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