Arteriosclerosis, Thrombosis, and Vascular Biology
JOURNAL OF THE AMERICAN HEART ASSOCIATION

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Arterioscler Thromb Vasc Biol 2007, 27:2763-2768: originally published online September 27, 2007
doi: 10.1161/ATVBAHA.107.152777
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 1079-5642. Online ISSN: 1524-4636

Soluble CD40 Ligand Predicts Ischemic Stroke and Myocardial Infarction in Patients With Nonvalvular Atrial Fibrillation

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Objective—Atrial fibrillation (AF) is associated with a high incidence of vascular disease that may be related to a prothrombotic and inflammatory state. Soluble CD40 ligand (sCD40L), which stems essentially from platelet activation, possesses inflammatory and prothrombotic properties. The aim of the study was to assess whether sCD40L is a predictor of stroke or myocardial infarction (MI) in patients with nonvalvular AF.

Methods and Results—Plasma levels of sCD40L were measured in 231 patients (177 [77%] had permanent or persistent AF, and 54 [23%] had paroxysmal AF). Patients were followed for a mean period of 27.8±8.8 months, and cardiovascular events such as fatal and nonfatal stroke and MI were recorded. AF population was divided in 2 groups according to sCD40L level above or below the median (4.76 ng/mL). The 2 patients' groups had similar distribution of cardiovascular risk factors, age, gender, medications, or serum C-reactive protein levels. During the follow-up period, vascular events occurred in 6 (2 nonfatal MI and 4 nonfatal ischemic strokes) of 116 patients with low levels of sCD40L (5.1%) and in 29 (11 fatal and 3 nonfatal MI; 3 fatal and 12 nonfatal ischemic strokes) of 115 patients with high levels (25.2%) (log-rank test: $P<0.001$). Using the COX proportional Hazards model, patients with sCD40L above the median were 4.63 times more likely to experience a vascular event (95% C.I.: 1.92 to 11.20).

Conclusions—This study shows that enhanced soluble CD40L level is a predictor of vascular events in patients with nonvalvular AF, thus suggesting that enhanced platelet activation may play a role in its clinical progression. **(*Arterioscler Thromb Vasc Biol*. 2007;27:2763-2768.)**

Key Words: atrial fibrillation ■ sCD40L ■ atherosclerosis

Nonvalvular atrial fibrillation (AF) is the most common cause of cardiac arrhythmia and is known to be associated with both thromboembolic and cardiovascular events.[1] In particular, patients with AF have about 5-fold increase of stroke risk, which is prevalently dependent on thrombosis occurring in the left atrium or left atrium appendage.[2] In addition to thromboembolic stroke patients with AF have a clinical history that is also complicated by other cardiovascular events including myocardial infarction (MI) and vascular death.[3] This is likely dependent on the fact that AF patients often show several risk factors for atherosclerosis such as hypertension and diabetes, which may favor the occurrence of coronary atherosclerosis and eventually MI.[4]

Oral anticoagulants or aspirin are established antithrombotic treatments to prevent thromboembolism in AF patients.[5] However, despite the fact that oral anticoagulants reduce by more than 50% the risk of stroke,[6] there is still a large number of patients, particularly those at high risk, who may not benefit from anticoagulant treatment. Therefore, identification of these patients, who are still at high risk of cardiovascular events despite antithrombotic treatment, could be useful to develop more appropriate prevention.

Platelet activation is a typical feature of patients with and at risk of developing cardiovascular events. Abnormal platelet activation has been observed in patients with AF particularly in case of coexistence of embolic or preembolic status.[7] This would suggest that platelet activation may be a marker of thromboembolic stroke in AF patients but the data so far reported are inconclusive.[8]

CD40 ligand (CD40L) is a protein of the tumor necrosis factor family that is implicated in the pathogenesis of atherosclerosis via its inflammatory and prothrombotic properties.[9–11] CD40L is expressed in hematopoietic cell types such as T lymphocytes, monocytes, or platelets and nonhematopoietic cells such as endothelial and smooth muscle cells.[9,12] A lot of attention has been recently given to the clinical

Original received July 27, 2007; final version accepted September 13, 2007.

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Arterioscler Thromb Vasc Biol **is available at http://atvb.ahajournals.org** **DOI: 10.1161/ATVBAHA.107.152777**

validity of soluble CD40L (sCD40L), which derives prevalently from platelet activation,[13] as a marker of cardiovascular events. Notably, sCD40L highly correlates with instable plaque and may be an important predictor of plaque instability and eventually plaque complication.[14,15] Observational and prospective studies supported this view by showing that sCD40L is a predictor of cardiovascular events including MI and stroke.[16–18]

There are several reasons that could render attractive the analysis of sCD40L in AF. In fact AF is associated with both platelet and clotting activation, which may be favored by CD40L[19] and potentially contribute to thrombus formation. Also, CD40L has inflammatory properties that could deteriorate the clinical course of AF by precipitating cardiovascular events. Accordingly, a previous study demonstrated that plasma levels of the inflammatory protein interleukin (IL) 6 predict vascular events in a population with AF.[20] Based on these findings we undertook a prospective study in which we analyzed if sCD40L predict cerebro- and cardiovascular events in a population with AF.

Methods

Two hundred thirty-one eligible subjects were recruited, between April 2001 and September 2006, from among consecutive patients admitted to the IV Division of Clinical Medicine because of nonvalvular AF—paroxysmal, persistent, or permanent (lasting >6 months). Of the 231 patients recruited, 177 (77%) had permanent or persistent AF, and 54 (23%) had paroxysmal AF.

Patient Selection

Patients were excluded if they had rheumatic AF; other cardiac reasons for exclusion were echocardiographic evidence of intracardiac thrombosis or tumor; left ventricular aneurysm, severe congestive heart failure (New York Heart Association functional class >3), or the presence of prosthetic valves; acute myocardial infarction or unstable angina during the previous month; carotid endarterectomy or coronary or peripheral revascularization procedures performed during the previous 6 months; acquired or congenital valvular disease (except mitral valve prolapse or mitral annulus calcification). The neurological exclusion criteria included CT brain scan evidence of cerebral hemorrhage, documented arteriovenous malformation or tumor, severe involutive cerebral disease, or the presence of a carotid lesion requiring surgical intervention.

According to ACC/AHA/ESC guidelines for the management of patients with atrial fibrillation, patients were classified as having low, moderate, or high risk for ischemic stroke.[5] Based on this classification 172 patients (74.4%) were treated with oral anticoagulants and 55 (23.8%) with aspirin (100 mg/d). Each patient gave informed consent to participate in the study, that was approved by the Ethical Committee.

Baseline

At entry, each patient's medical history was taken and he/she underwent a physical examination. The following diagnostic procedures were also performed: routine blood laboratory tests, baseline 12-lead ECG, M-mode, and 2-dimensional echocardiography with echocolor-doppler, echocolor-doppler of the supra-aortic trunks, and chest roentgenography.

Follow-Up

Patients were periodically monitored (every 3 months) to assess compliance and clinical status, ECG, and laboratory tests (anticoagulant treatment was adjusted to ensure INR values within the range of 2.0 to 3.0), and a record was made of the occurrence of any outcome events, the degree of disability, the appearance of any adverse drug reactions, and the patient's compliance with the prescribed drugs. All deaths and their causes were recorded, with death certificates being obtained in the case of deaths occurring outside the hospital.

Outcome Events

The combined incidence of the following vascular events was considered the primary outcome of the study: nonfatal and fatal stroke, fatal and nonfatal myocardial infarction. A diagnosis of myocardial infarction required at least 2 of the following criteria: history of chest discomfort, development of a pathological Q wave on ECG tracings, and elevation of specific cardiac enzymes to values of more than twice the upper normal limit. The occurrence of stroke was determined on the basis of clinical manifestations and confirmed by CT. If a patient died within 4 weeks of an acute vascular event, this was recorded as cardiovascular death attributable to an ischemic event.

Blood Collection and Laboratory Analysis

Blood sample was withdrawn without stasis to minimize platelet activation from subjects who had fasted for 12 hours, directly mixed in a vacutainer (Vacutainer Systems, Belliver Industrial Estate) with 1 part of 3,8% Na citrate (ratio 9:1) and immediately centrifuged for 20 minutes at 2000*g* at −4°C. Plasma samples were stored at −80°C until use.

Plasma levels of sCD40L were evaluated behind an immunoassay (Quantikine CD40 ligand, R&D Systems). Intraassay and interassay coefficients of variation were 6% and 7%, respectively.

Serum high-sensitivity C-reactive protein (CRP) was assayed by an immunonephelometric method (N-High Sensitivity CRP, Dade Behring Marburg Gmbh). Intra- and interassay coefficients of variation were 3 and 2.5%, respectively.

Statistical Analysis

Two-sided *t* test was used to compare means. Results were confirmed by nonparametric tests as Wilcoxon rank sum. Pearson chi-square test was used to compare proportions.

To evaluate whether high levels of sCD40L were associated with different degree of ischemic risk, the AF population was divided into 2 groups: patients above or below the median (4.76 ng/mL). The cumulative risk of vascular events (stroke and myocardial infarction) within each group was estimated through the Kaplan–Meier method. The survival curves of the 2 groups were then formally compared using the log-rank test. The validity of constant incidence ratios over the follow-up was checked using Nelson-Aalen cumulative hazard estimates.[21] Kaplan–Meier survival analysis was performed also to compare the survival curves of patients in the highest quartile of sCD40L versus patients of the other quartiles.

Cox proportional hazards analysis was used to calculate the adjusted relative hazards of vascular events by each clinical variable. The following variables, assessed at the baseline evaluation, were considered as potential predictors of ischemic events: age, male, sex, hypertension, diabetes, dyslipidemia, oral anticoagulant treatment, antiplatelet treatment, previous cerebral ischemia, previous coronary heart disease, CRP (above the median), and sCD40L >4.76 ng/mL. Stochastic level of entry into the model was set at 0.10, and interaction terms were explored for all variables in the final model. A minimum events-to-variable ratio of 10 was maintained in multivariate modeling to avoid overfitting, and Schoenfeld's test was performed to check the validity of proportional hazards assumption.[22] Cox analyses were performed for all vascular events, as well as separately for cardio- and cerebrovascular events.

A probability value of <0.05 was considered significant for all analyses, which were performed using STATA statistical software version 8.0 (STATA corporation, College Station, TX).

Results

The mean age of the AF population was 72.4±10.3 years, and 190 subjects (82.2%) were older than 65 years of age. Half of the patients were male (48%). History of stroke and

Table 1. Baseline Characteristics of Patients With Atrial Fibrillation According to the Level of Soluble CD40 Ligand

Characteristics	Low Level of Soluble CD40 Ligand (≤4.76 ng/mL)	High Level of Soluble CD40 Ligand (>4.76 ng/mL)	*P* Value
No. of patients	116	115	...
Male sex (%)	50 (43.1)	61 (53.0)	0.131
Mean age, y	71.3±11.2	73.5±9.3	0.103
Coronary artery disease	14 (12.1)	29 (25.2)	0.010
History of stroke or TIA	4 (3.5)	26 (22.6)	<0.001
Cardiovascular risk factors (%)			
Diabetes	17 (14.7)	20 (17.4)	0.571
Hypercholesterolemia	28 (24.1)	35 (30.4)	0.283
Hypertension	87 (75.0)	92 (80.0)	0.363
Current smoking	13 (11.2)	9 (7.8)	0.381
Risk stratification for ischemic (%)			
High	88 (75.8)	97 (84.3)	0.800
Intermediate	18 (15.5)	14 (12.1)	0.586
Low	10 (8.6)	4 (3.4)	0.173
Medications at discharge (%)			
Aspirin	31 (26.7)	24 (20.8)	0.373
Oral Anticoagulants	82 (70.6)	90 (78.2)	0.243
Statins	26 (22.4)	59 (51.3)	<0.001
ACE inhibitors	55 (47.4)	63 (54.7)	0.323
Angiotensin type-1 receptor blockers	12 (10.3)	16 (13.9)	0.529
Calcium antagonists	56 (48.2)	52 (45.2)	0.641
Beta blockers	35 (30.1)	33 (28.6)	0.919
Diuretics	70 (60.3)	79 (68.1)	0.235
CRP (mg/L)*	3.65 (2.9–7.6)	3.9 (2.9–13.2)	0.400
Soluble CD40 ligand (ng/mL)	3.4±0.7	6.6±1.6	<0.001

*Data expressed as median and interquartile range.

coronary heart disease was reported by 12.9% and 18.6% of patients, respectively. About 73% of patients were receiving antihypertensive treatment (1 or more medication): diuretics (64.5%), ACE-inhibitors (51.0%), Angiotensin-renin blockers (12.1%), calcium-antagonists (46.7%), and β-blockers (29.4%).

To evaluate whether elevated levels of sCD40L indicated an increased risk of cardiovascular and cerebrovascular events, the AF population was divided into 2 groups on the ground of the median value of sCD40L (4.76 ng/mL). The 2 groups had a similar distribution of cardiovascular risk factors, age, gender, and clinical and laboratory markers of risk factors; also, antiplatelet and anticoagulant drugs were equally distributed between the 2 groups. Conversely, statin treatment was more frequent in the group with high level of sCD40L. The clinical history of previous stroke or coronary heart disease was significantly higher in patients with levels of sCD40L above the median (Table 1).

Patients were followed for a mean period of 27.8±8.8 months, yielding a total of 6434 person-months of observation. The follow-up period was not different in the 2 study groups (28.6±7.6 and 27.1±9.8 months in patients with low and high levels of sCD40L, respectively).

During the follow-up 35 patients (15.1%) experienced cardiovascular and cerebrovascular events; 19 (8.2%) had ischemic stroke, and 16 (6.9%) had MI. Vascular events occurred in 6 (2 nonfatal MI and 4 nonfatal ischemic strokes) of 116 patients with low levels of sCD40L (5.1%) and in 29 (11 fatal and 3 nonfatal MI; 3 fatal and 12 nonfatal ischemic strokes) of 115 patients with high levels (25.2%).

Levels of sCD40L above the median were significantly associated with shorter time to ischemic events (log-rank test: $P<0.001$; Figure 1). The association between high levels of sCD40L and shorter time to ischemic events was even more evident in the comparative evaluation of patients in the highest quartile versus the others (log-rank test: $P<0.0005$; Figure 2).

The incidence rate of vascular events was 0.93 and 0.18 per 100 months of observation in patients with sCD40L levels above and below the median, respectively. Using the Cox proportional hazards model, after controlling for other variables, patients with sCD40L levels above the median were 4.63 times more likely to experience a vascular event (95% C.I.: 1.92 to 11.20). The adjusted risk of vascular events among patients with diabetes was 2.24 (95% C.I.:1.07 to 4.70; Table 2). No significant interactions were detected and therefore were not included in the final model. High levels of sCD40L and diabetes were independently related to vascular events also when the Cox analysis was performed comparing

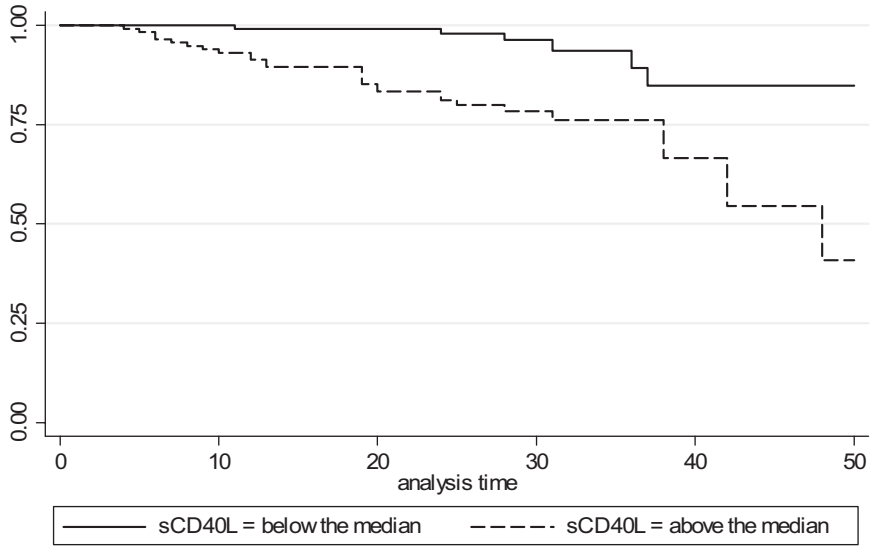

Figure 1. Kaplan–Meier estimates of time to ischemic events by levels of sCD40L (below and above the median).

patients in the highest quartile of sCD40L levels versus the other (data not shown).

Cox analyses were performed also separately for cardiovascular and cerebrovascular events. Patients with sCD40L levels above the median were 6.58 times more likely to experience cardiovascular events (95% C.I.:1.49 to 29.11), whereas the adjusted risk in patients with diabetes was 3.77 (95% C.I.:1.34 to 10.65). High levels of sCD40L, but not diabetes, was related to cerebrovascular events (HR=3.28; 95% C.I.:1.27 to 11.53), and the risk of cerebrovascular events increased significantly with age (HR=1.07; 95% C.I.:1.01 to 1.14).

Discussion

This study provides evidence that plasma levels of sCD40L are predictive of cardiovascular events including ischemic stroke and MI in patients with AF.

The AF cohort screened encompassed prevalently high-risk patients as more than 70% of patients had to be treated with oral anticoagulants. During the follow-up of 3 years such population experienced 8.2% ischemic stroke. This finding is consistent with previous data showing that in patients treated with adjusted-dosed warfarin stroke incidences range from 1.1% to 4% per year.[23–26] In addition to ischemic stroke, our population also suffered from coronary events with an incidence rate of 6.9% during the observed period. Also this finding is consistent with previous studies showing that a relatively high percentage of patients with AF may suffer from MI. The rate we observed is a little bit more than that found in previous studies, where MI rate ranged from 0.6% to 1.4% per year[23,24] but relatively close to an Italian study in NVAF patients in which the annual rate of MI was 3.7%.[26]

Previous studies performed in patients with established acute or chronic coronary heart disease documented that plasma levels of sCD40L are predictive of atherosclerotic progression.[16,17,27] Such relationships seem to be relevant also in patients with carotid atherosclerosis and in those with obstructive sleep apnea.[28,29]

In the present study we analyzed the relevance of sCD40L as predictor of cardiovascular events in AF patients. Herewith, we demonstrated that patients with sCD40L above 4.7 ng/mL are at higher risk of ischemic stroke and MI compared

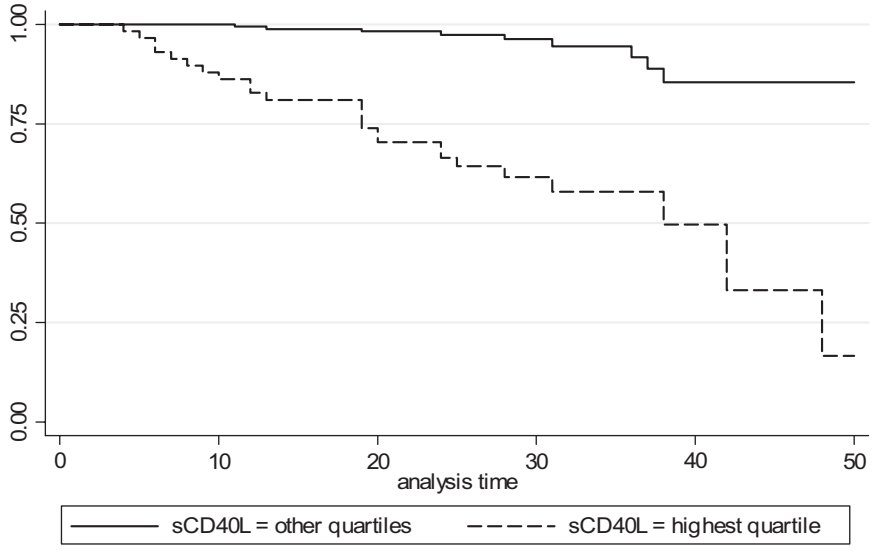

Figure 2. Kaplan–Meier estimates of time to ischemic events by levels of sCD40L (highest quartile vs other quartiles).

Table 2. Adjusted Hazard Ratios of Vascular Events According to Selected Variables

Variables	HR*	95% C.I.	*P* Value
Soluble CD40 ligand levels above the median (4.76 ng/mL)	4.63	1.91–11.1	0.001
Diabetes	2.24	1.06–4.70	0.033
Age	1.03	0.99–1.07	0.089

Based on a Cox proportional Hazards model (n=231; LR chi-square=24.8; Schoenfeld global test *P*=0.19).

*Hazard ratio>1 favors vascular events.

with those with lower values. Such a relationship was independent from other recognized risk factors such as age, hypertension, diabetes, or previous history of cardiovascular events. Another interesting finding of the study was that sCD40L was an independent predictor of either ischemic stroke or MI; however, because of the relatively small sample size and the limited number of events such observation should be wisely considered. It is intriguing, however, that this molecule predicted 2 events that are prevalently caused by different mechanisms: thromboembolic in the case of ischemic stroke and atherothrombotic in the case of MI.

To exclude the existence of confounding factors related to concomitant treatments, we checked for distribution of medications that could affect sCD40L. Aspirin, for instance, has been reported to reduce sCD40L.[30] However, patients on aspirin treatment were equally distributed between groups with high or low sCD40L levels. We found, on the contrary, a higher percentage of statin treatment in patients with high sCD40L levels. Therefore, we cannot exclude that this could have underestimated the predictive value of sCD40L as statins have been shown to reduce sCD40L.[31,32] Most patients in our cohort were on an oral anticoagulant treatment. However, we have previously found that oral anticoagulants did not affect sCD40L in patients with lupus anticoagulant.[33]

Our data are apparently at variance with a recent study by Lip et al[34] which did not find any relationship between sCD40L and cardiovascular events in a population with AF. Different population and methodology of the study may perhaps account for such divergence. Whereas our cohort included prevalently patients at high risk of cardiovascular events, most patients in the Lip's trial were on treatment with aspirin. Therefore, we cannot exclude that sCD40L is not useful to predict cardiovascular events in a population at low risk. This hypothesis is consistent with another study performed in a large apparently healthy population (the Dallas trial) demonstrating that sCD40L is not useful to screen for subclinical atherosclerosis in patients at low risk.[35] Also, duration of observation could have had a different impact on the prognostic value of sCD40L, because our follow-up (about 3 years) was much longer than that of the Lip's study (1 year).

Circulating levels of sCD40L are considered a marker of platelet activation as more than 95% seems to be of platelet origin.[13] The reason why sC40L is more useful in predicting cardiovascular events in this setting compared with other molecules such as p-selectin[36,37] may depend on the peculiarity of its prothrombotic property. Thus, CD40L is able to promote overexpression of tissue factor, a glycoprotein that has a crucial role in the activation of coagulation cascade,[38] and is implicated in the process of platelet aggregation via binding to platelet glycoprotein (GP) IIb/IIIa.[39] In virtue of these biological characteristics, sCD40L are likely to reflect both clotting activation and platelet aggregation occurring in patients with AF and, hence, could better identify patients more prone to cardiovascular complications.

AF is associated with an inflammatory state that may concur in favoring cardiovascular complication. Plasma levels of IL-6 are elevated in this setting and may predict cardiovascular events.[20] CD40L also has inflammatory property including expression of adhesive molecule, chemokines, and metalloproteinases,[14] and this adds further to the possibility that inflammation may predict poor cardiovascular outcome in AF patients. Consistent with previous studies[16,35] we found no relationship between CD40L and CRP indicating that they likely represent 2 different pathways of the inflammatory status.

The study has potential limitation and implication. The predictive value of sCD40L was calculated by combining 2 end points with probable different mechanism of disease, ie, ischemic stroke and MI. Thus, it is unclear from our study whether sCD40L is sensitive to vascular events of thromboembolic or atherotrombotic origin. Larger sample size is, therefore, necessary to evaluate whether sCD40L is predictive of either ischemic stroke or MI.

A recent study in elderly population with AF confirmed that, despite oral anticoagulants, these patients had a 5.9% annual risk of major outcomes including stroke, MI, and vascular death.[40] This indicates that other therapeutic strategies should be implemented to reduce such high risk. Our data suggest to investigate whether sCD40L lowering by appropriate therapy may be associated with a further reduction of cardiovascular events in elderly AF population.

In conclusion this study shows that sCD40L is able to predict cardiovascular events including ischemic stroke and MI in an AF population at high risk. These data cannot be extrapolated to a population at low risk and further study is necessary to investigate this issue. Analysis of this biomarker may represent another approach to stratify the cardiovascular risk in AF population.

Sources of Funding

This study was supported in part by a grant from the University of Rome "La Sapienza" (Ateneo 2005).

Disclosures

None.

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