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A score including ADAM17 substrates correlates to recurring cardiovascular event in subjects with atherosclerosis

Rizza Stefano [a,b], Copetti Massimiliano [c], Cardellini Marina [a,b], Menghini Rossella [a], Pecchioli Chiara [a,b], Luzi Alessio [a,b], Di Cola Giovanni [a,b], Porzio Ottavia [d], Ippoliti Arnaldo [e], Romeo Franco [a], Pellegrini Fabio [c], Federici Massimo [a,b,*]

[a] Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy
[b] Center for Atherosclerosis, Policlinico Tor Vergata, Rome, Italy
[c] Unit of Biostatistics, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy
[d] Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy
[e] Department of Biopathology and Diagnostic Imaging, University of Rome Tor Vergata, Italy

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ABSTRACT

Objective: Atherosclerosis disease is a leading cause for mortality and morbidity. The narrowing/rupture of a vulnerable atherosclerotic plaque is accountable for acute cardiovascular events. However, despite of an intensive research, a reliable clinical method which may disclose a vulnerable patient is still unavailable.

Approach and results: We tested the association of ADAM17 (A Disintegrin and Metallo Protease Domain 17) circulating substrates (sICAM-1, sVCAM-1, sIL6R and sTNFR1) with a second major cardiovascular events [MACEs] (cardiovascular death, peripheral artery surgeries, non-fatal myocardial infarction and non-fatal stroke) in 298 patients belonging to the Vascular Diabetes (AVD) study. To evaluate ADAM17 activity we create ADAM17 score through a RECPAM model. Finally we tested the discrimination ability and the reclassification of clinical models.

At follow-up (mean 47 months, range 1–118 months), 55 MACEs occurred (14 nonfatal MI, 14 nonfatal strokes, 17 peripheral artery procedures and 10 cardiovascular deaths) (incidence = 7.8% person-years). An increased risk for incident events was observed among the high ADAM17 score individuals both in univariable (HR 19.20, 95% CI 15.82–63.36, $p < 0.001$) and multivariable analysis (HR 3.42, 95% CI 1.55–7.54, $p < 0.001$). Finally we found that ADAM17 score significantly increases the prediction accuracy of the Framingham *Recurring-Coronary-Heart-Disease-Score*, with a significant improvement in discrimination (integrated discrimination improvement = 9%, $p = 0.012$) and correctly reclassifying 10% of events and 41% of non-events resulting in a cNRI = 0.51 ($p = 0.005$).

Conclusion: We demonstrated a positive role of ADAM17 activity to predicting CV events. We think that an approach that targets strategies beyond classic cardiovascular risk factors control is necessary in individuals with an established vascular atherosclerosis.

1. Introduction

Atherosclerosis is the primary cause for coronary artery disease and stroke in the Western World [1].

There is an evolving debate about the value of assessing levels of soluble biomarkers for the prediction of plaques ruptures [2,3]. The challenging point is therefore to identify a soluble biomarker which may be helpful to identify vulnerable patients that despite no clinical evidence of atherosclerosis and no concomitance of several cardiovascular risk factors are very likely to be affected by

List of abbreviations: ADAM17, A Disintegrin and Metallo Protease Domain 17; MACEs, Major cardiovascular events; CV, Cardiovascular; TIMP3, Tissue inhibitor of metalloproteinase 3; AVD, Athero vascular diabetes; RECPAM, Recursive Partitioning and Amalgamation; RCHD, Recurrent Coronary Heart Disease; IDI, Integrated Discrimination Improvement; RIDI, Relative Integrated Discrimination Improvement; NRI, Net reclassification improvement; PTCA, Transluminal coronary angioplasty; CEA, Carotid endo-arterectomy; OGTT, Oral Glucose Tolerance Test.

* Corresponding author. Center for Atherosclerosis, Department of Systems Medicine, University of Rome Tor Vergata, Via Montpellier 1, 00133 Rome, Italy.
E-mail address: federicm@uniroma2.it (F. Massimo).

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subclinical frail atherosclerotic plaques.

The TNF-alpha Converting Enzyme (TACE), also called A Disintegrin and Metallo Protease Domain 17 (ADAM17), is a type I transmembrane protein that belongs to a superfamily of Zn dependent metalloproteases. ADAM17 plays a key role in the regulation of the proteolytic release from cellular membranes of some adhesion molecules, cytokines, chemokines, growth factors and their receptors, including ICAM-1, VCAM-1, TNF-α, TNF receptors I and II, TGF-a, L-selectin, IL-6 receptor and M-CSF receptor 1, affecting downstream signaling and cellular responses [4].

ADAM17 activity is involved in major acute and chronic inflammatory and degenerative diseases such as cancer, inflammatory diseases, Alzheimer and atherosclerosis [4] Interestingly, it has been described that a local ADAM17 activity is correlated to adverse clinical outcomes in acute coronary atherosclerosis setting [5]. Similarly we and others have demonstrated that an unbalanced ADAM 17/tissue inhibitor of metalloproteinase 3 (TIMP3) ratio is characteristic of unstable carotid plaques in diabetic and non diabetic subjects [6,7].

TIMP3 is also an inhibitor of metalloproteinases (MMP) 9 and 14 both involved in plaque destabilization in previous studies [6–8].

Here, we tested whether ADAM17 substrates included in a new single score are associated with cardiovascular events.

Secondarily, we tried to quantify the prediction improvement of a classic algorithm risk score when the assessment of the circulating ADAM17 substrates was added.

2. Materials and methods

2.1. Assessment of ADAM17 substrates

Serum samples were analyzed by a sandwich enzyme-linked immunosorbent assay (ELISA) method using antimouse antibodies for human soluble tumor necrosis factor receptor 1 (sTNFR1), soluble interleukin 6 receptor (sIL6R), soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble intercellular cell adhesion molecule 1 (sICAM-1) (all from R&D Systems, Minneapolis, MN) according to manufacturer's instructions.

2.2. Study participants

We evaluated baseline ADAM17 activity by dosing its four main circulating substrates levels (sVCAM-1, sICAM-1, sIL6R, sTNFR1) in 298 consecutive participants belonging to the Athero-Vascular Diabetes (AVD) Study which has been described in detail previously [9]. This study was approved by the University of Tor Vergata Institutional Review Board and carried out in accordance with the principles of the Declaration Of Helsinki as revised in 2000.

2.3. Follow-up

The patients had a routine follow-up at six months and one year after inclusion, then yearly thereafter. Follow-up outcomes included the development of one major cardiovascular event such as nonfatal stroke, nonfatal myocardial infarction, peripheral vascular surgical procedures and cardiovascular death (fatal stroke or fatal myocardial infarction). Information on nonfatal events was sought from study participants by telephone interviews and confirmed by review of hospital records. In case of fatal events, information was obtained from their primary care physicians or from death certificates.

2.4. Statistical methods

Baseline patients' clinical characteristics were reported as mean and standard deviation or frequencies and percentages for continuous and categorical variables, respectively.

Univariable and multivariable time to event analysis were performed using Cox proportional hazards regression model. Risks were reported as hazard ratios (HR) along their 95% confidence interval (95% CI).

The clinical predictive ADAM17 score and the Reclassification methods are described in the online supplementary methods.

A p-value <0.05 will be considered as significant. All analyses will be performed using SAS Release 9.3 (SAS Institute, Cary, NC).

3. Results

During the follow-up (mean 47 months, range 1–118 months) 55 major adverse cardiovascular events (MACEs) occurred: 14 nonfatal MI, 14 nonfatal strokes, 17 peripheral artery procedures and 10 cardiovascular deaths (incidence of MACEs was 7.8% person-years). Peripheral artery procedures included 8 PTCA (transluminal coronary angioplasty), 5 CEA (carotid endo-arterectomy) and 4 peripheral stenting placements.

The clinical characteristics of study population according to event or no event are summarized in Table 1. Of note, no patients reported a history of diagnosed diabetes [9]. Therefore, according to the inclusion criteria, all individuals included into the study underwent an Oral Glucose Tolerance Test (OGTT) to detect an unknown diabetes. Consequently, patients with unknown diabetes did not take any specific therapeutic agents. Finally no significant differences in pharmacological therapy emerged between the group of individuals who had a MACES and the group of participants who had not (On-line Table 1).

Crude and age-sex-BMI adjusted second cardiovascular event HRs are reported in Table 2 for the main demographic, clinical and biochemical features.

To create a quantitative risk score which may illustrate the ADAM17 activity on the CV event, we applied a RECPAM model to the circulating levels of ADAM17 substrates (sVCAM-1, sICAM-1, sIL6R, sTNFR1) expressed in tertiles (Fig. 1). The model excluded s-IL-6-r because not significantly associated to the event in such a modeling. RECPAM identified 3 homogeneous subgroups of patients in terms of event rate according to specific interactions between s-TNFα-rec, sVCAM-1 and sICAM-1 levels. Class 1 included the participants with the lower risk to develop the event whereas Class 3 involved patients with the higher risk (HR = 6.69, CI 95%

Table 1
Clinical characteristics of study population.

	Patients without MACEs (n°243)	Patients with MACEs (n°55)
Age (y)	58.19 ± 17.93	66.73 ± 9.94
Sex (m/f)	148/95	44/11
Smokers or ex smokers (n, %)	172 (71%)	51 (94%)
BMI	26.53 ± 4.13	24.43 ± 4.27
Diast BP (mmHg)	80.56 ± 10.60	80.56 ± 11.84
Syst BP (mmHg)	134.03 ± 22.61	137.59 ± 20.92
Diabetes (n, %)	44 (18%)	11 (20%)
A1c (%)	5.51 ± 0.42	5.69 ± 0.59
Tot cholesterol (mg/dl)	184.29 ± 37.69	173.33 ± 47.34
LDL cholesterol (mg/dl)	116.33 ± 32.89	109.20 ± 38.49
HDL cholesterol (mg/dl)	49.96 ± 14.33	45.98 ± 13.94
Triglycerides (mg/dl)	124.99 ± 67.00	138.44 ± 67.33
sICAM-1 (ng/ml)	285.28 ± 113.79	357.90 ± 171.60
sVCAM-1 (ng/ml)	732.94 ± 386.63	954.97 ± 393.73
sIL6R (ng/ml)	40.33 ± 12.94	43.06 ± 10.35
sTNFR1 (pg/ml)	1.60 ± 0.57	1.83 ± 0.71
Follow-up (months)	57.71 ± 22.19	32.68 ± 19.98

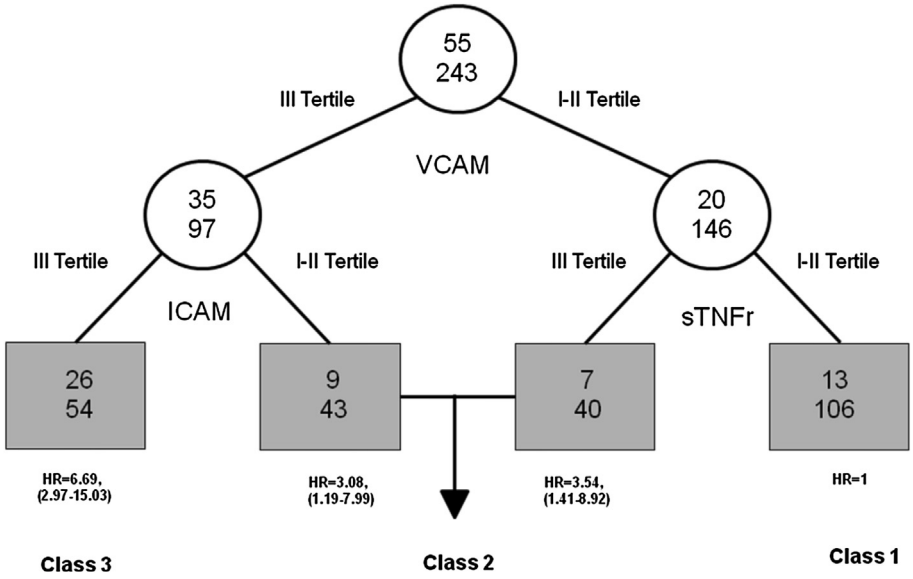

Fig. 1. Figure shows patients with different MACE risks based on ADAM17 substrates interactions that were identified by the REPCAM analysis. The tree-growing algorithm estimates hazard ratios from a Cox proportional hazards regression model with sVCAM-1, sICAM-1, sIL6R and sTNFR1 as candidate splitting variables. Chosen splitting variables are shown between branches, while condition sending patients to left or right sibling is on relative branch. Results were reported as HR along with their 95%CI. Class 1 with the lowest MACE rate was reference category (HR = 1). Circles indicate subgroups of patients. Squares indicate patient subgroup RECPAM class. Numbers inside circles and squares represent the number of events (top) and the number of non-events (bottom), respectively.

2.97–15.03). Subjects with higher cardiovascular risk (high ADAM17 score level) have the higher tertile of sICAM-1 and the higher tertile of sVCAM-1. On the opposite side, patients with lower cardiovascular risk (low ADAM17 score level) have the lower or medium tertiles of sVCAM-1 and lower or medium tertiles of sTNFR1. All the other individuals have an intermediate risk (medium ADAM17 score level).

In the time to event analysis (Table 2), respect to low ADAM17 score participants, an increased risk for incident events was observed among the high ADAM17 score individuals (KM curves shown in Fig. 2 for display only) both in univariable (HR 19.20, 95% CI 15.82–63.36, $p < 0.001$) and multivariable analysis (HR 3.42, 95% CI 1.55–7.54, $p < 0.001$) after adjusting for age, sex and BMI.

Next, we tested if the addition of ADAM17 score to the Framingham Recurring Coronary Heart Disease (RCHD) model [10]. It is a well established predictive risk model; thus we assessed if the addition of ADAM17 score could improve the 4-year discriminative prediction of MACEs. We found that ADAM17 score significantly increases the prediction accuracy. The survival C-statistic of RCHD score was 0.74 (95%CI = 0.67–0.81) and such a model resulted calibrated (HL, $p = 0.91$). Addition of the ADAM17 score into this model (calibration HL, $p = 0.95$) led to significant increase of

Table 2
Univariable and multivariable associations between clinical parameters and MACEs.

Variable	Univariable analysis			Multivariable analysis[a]		
	HR	95% CI	p	HR	95% CI	p
Sex						
Male	3.023	1.561–5.853	0.001	4.326	0.947–19.766	0.059
Female	1			1		
Age	1.031	1.013–1.050	<0.001	1.011	0.954–1.071	0.713
BMI	1.052	0.991–1.116	0.097	1.123	0.848–1.487	0.417
Smoke						
Ex or current	3.665	1.846–7.275	<0.001	1.05	0.285–3.867	0.942
Never	1			1		
Systolic BP	1.006	0.994–1.017	0.332	1.007	0.971–1.043	0.718
Diastolic BP	1.002	0.978–1.027	0.855	0.99	0.919–1.065	0.779
TOT Cholesterol	0.993	0.987–1.000	0.054	0.997	0.943–1.054	0.924
HDL Chol	0.977	0.958–0.997	0.026	1.007	0.948–1.069	0.829
LDL Chol	0.994	0.986–1.002	0.12	0.982	0.929–1.038	0.511
Triglycerides	1.003	1.000–1.006	0.083	0.969	0.942–0.996	0.025
A1c	2.202	1.346–3.601	0.002	4.505	0.810–25.068	0.086
sIL6R	1.016	0.997–1.036	0.099	0.76	0.580–0.996	0.047
sTNFR1	1.7	1.152–2.507	0.007	22.255	1.79–276.76	0.016
sVCAM1	1.001	1.001–1.001	<0.001	1.001	0.997–1.005	0.692
sICAM-1	1.003	1.002–1.004	<0.001	1.003	0.994–1.013	0.512
ADAM17 score						
High ADAM17 score	19.2	5.819–63.361	<0.001	3.415	1.548–7.536	0.002
Medium ADAM17 score	12.52	3.737–41.933	<0.001	1.918	0.857–4.291	0.113
Low ADAM17 score	1			1		

[a] Adjusting for patient's age, sex and BMI.

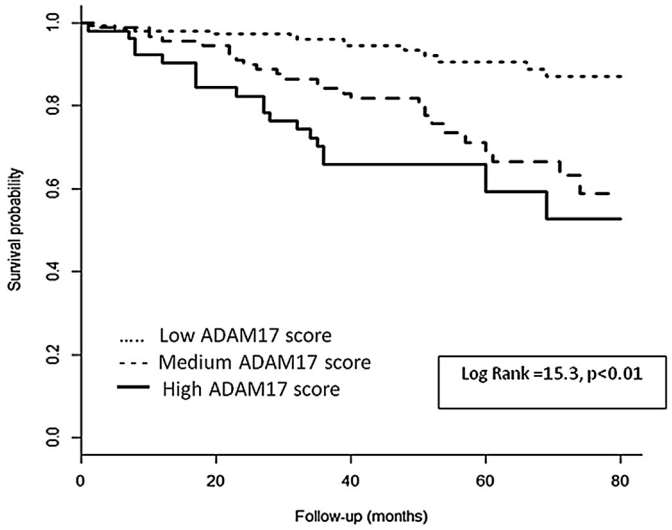

Fig. 2. Kaplan–Meier curves for ADAM17 score classes. Log Rank: 15.3, $p < 0.01$.

survival C-statistic ($p = 0.018$) to 0.80 (95%CI = 0.70–0.85) as well as statistically significant improvement in discrimination evaluated by IDI = 9% (95%CI = 4%–14%, $p = 0.012$) and RIDI = 39% (95%CI: 7%–74%, $p = 0.013$). Moreover, the introduction of ADAM17 score into the model correctly reclassified 10% of events and 41% of non-events resulting in a cNRI = 0.51 (95%CI = 0.10–0.79, $p = 0.005$).

4. Discussion

During the past decade the identification of atherosclerotic vulnerable plaques, likely to cause an acute event in the future, has been a topic of intensive research. Several noninvasive modalities has been evaluated, but, unfortunately, a method that can properly assess all the aspects of plaque and based on non invasive approach either at imaging or biochemical level, is still unavailable [11]. We found that increased circulating levels of ADAM17 substrates were associated to a significant higher rate of second cardiovascular events in a cohort of patients with established vascular atherosclerosis. Although we have not explored ADAM17 protease activity directly *in vivo* it is conceivable that the increase of its substrates in the circulation depends from increased enzymatic activity at local inflammatory sites. Therefore, to study ADAM17 activity in an atherosclerotic setting we decided to evaluate, among its many substrates [12,13], those that were already independently implicated in severity or complications of atherosclerosis [14], but never with a combined approach. We reasoned that local over-activity of ADAM17 may weaken atherosclerotic plaque, causing their rupture, as a consequence of enzyme activation promoted by well known cardiovascular risk factors such as hyperglycemia, hyperinsulinemia and oxidized LDL and consequent increase in intraplaque inflammatory cells trafficking [7,14–16].

We have chosen to analyze ADAM17 substrates since they also unify the effect of different risk factors (such as insulin resistance/diabetes) on endothelial function and the consequent effect on atherosclerosis plaque evolution [17–20].

To explore ADAM17 *in vivo* activity we created a simple 3 levels score by RECPAM model that was able to identify distinct and homogeneous subgroups of individuals in terms of event rate and, therefore, those with very high cardiovascular risk. Our ADAM17 score is composed by circulating levels of sTNFR1, sVCAM-1, sICAM-1 but not sIL6R because the latter was not significantly associated to the events. This is surprising since sIL6R has been related to cardiovascular mortality [21]. However, it has been recently described that the most common functional variant in *IL6R* (Asp358Ala) is unrelated to the common CVD risk factors and it is associated in a dose-dependent manner with a decreased concentration of C-reactive protein and fibrinogen, and might consequently dampen the systemic inflammation [22]. This result supports the hypothesis that IL6R may play different roles depending on the different genetic variant.

The Framingham risk score remains the most widely used tool for assessment of risk prediction of CV events [23]. Conventional risk factors in the Framingham risk score such as age, male sex, hypercholesterolemia, hypertension, and smoking, account for most of the risk of CVD and have been the basis of risk assessment for decades. However, approximately one-third of individuals with 0 or 1 risk factor develop coronary heart diseases [24,25] and up to 40% of individuals with cholesterol levels below the population average die from CHD [26]. Furthermore, many CV events occur in patients treated with statin therapy.

As such, a wide list of biomarkers, genetic polymorphism arrays, and direct imaging of subclinical atherosclerosis with coronary artery calcium or carotid intima-media thickness have been investigated for refinement of risk assessment and preventive therapy allocation.

Individuals in secondary prevention have already a diffuse vascular atherosclerosis; therefore the use of a risk algorithm which does not include the plaques biology evaluation, or at least a surrogate, is a particular limitation. In fact, using only the classic cardiovascular risk factors we treat at the same manner patients with an initial vascular inflammation and individuals with an overt cardiovascular atherosclerosis. Differently, our data, including endothelial activation/inflammatory markers, may increase our ability to identify the vulnerable patient. Obviously other factors pointing to assess the thrombotic potential or the digestion of the necrotic core in the atherosclerotic plaque might be added in future studies.

We found a significant association between ADAM17 score and CV events both in univariable and multivariable analysis. To confirm the strength of our finding we incorporated the ADAM17 score into Framingham RCHD score [10] to improve its ability in predicting a second CV event. ADAM17 score substantively improved the Framingham RCHD. We showed a significant discrimination and reclassification improvement for CV events when ADAM17 score was assessed in addition to classical risk factors. The combined incorporation of the 3 biomarkers as a single covariate improved the c-statistic and reclassification metrics. Our result is statistically significant but rather faint. This point may be an important limitation but certainly is not surprising. In fact it has been already demonstrated that in particular setting of patients the incorporation of external risk factors into the Framingham score improves global CVD risk prediction, and when it happens, this improvement is frequently modest [27,28] even when the added biomarker (hsCRP) is strongly associated to CV risk [29]. Noteworthy the observed improvement is not only statistically significant but also clinically relevant allowing correct reclassification of large proportion of patients both with and without events. Nevertheless, our present finding needs replication in large prospective studies before it can be considered as established.

Our results have some limitations. First, the lack of a second cohort for an independent validation, although a replication of our finding is not easy because of the very particular vascular condition of the study population. Every patient had a non fatal MACE and no apparent record of DM2. However, to strengthen our results we adopted a permutation analysis for RECPAM model and used a bootstrap approach for the reclassification metrics, thus avoiding to produce over-optimistic results and obtaining a robust internal

validation.

Second, we acknowledge that a composite end-point comprising different cardiovascular events may be related to different pathogenic backgrounds. We cannot exclude, in fact, that some severe events may be unrelated to "vulnerable plaques" such as embolic stroke or a peripheral vascular surgical procedures for a slow narrowing of an atherosclerotic plaque. Though we recognized that further studies using unique clinical events as independent endpoints are likely to allow a deeper understanding of the biology of atherosclerotic plaque vulnerability, our results suggest that the use of ADAM17 substrates altogether may be a short come to trace the increased activity of the inflammatory and remodeling processes involved in plaque destabilization [30,31]. Whether the predictive role of ADAM17 score may be extended to clinical situations involving patients with a more moderate cardiovascular risk remains to be determined.

5. Significance

Our results suggest that measuring ADAM17 activity may help to predict major cardiovascular events in subjects with an established vascular atherosclerosis. To evaluate ADAM17 activity we dosed its four main substrates levels (sVCAM-1, sICAM-1, sIL6R, sTNFR1) in 298 consecutive participants with an established clinical vascular atherosclerosis, who were followed for MACEs (mean 47 months, range 1–118 months). To create a quantitative risk score which may illustrate the ADAM17 activity on the CV event we applied a RECPAM model. RECPAM identified 3 homogeneous subgroups of patients in terms of event risk.

An increased risk for incident events was observed among the high ADAM17 score individuals both in univariable and multivariable analysis. Finally we showed a significant enhancement of the Framingham risk score by adding ADAM17 activity to the classical risk factors. We conclude that in individuals with an established vascular atherosclerosis an approach that targets strategies beyond classic cardiovascular risk factors control is helpful.

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Disclosures

Authors declare that they have neither financial nor competing interests.

Conflict of interest

Authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2015.01.029.

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