IgA anticardiolipin antibody is associated with the extent of daily-life ischaemia in patients with chronic coronary artery disease

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Background: Circulating anticardiolipin antibodies (aCL) may cause endothelial dysfunction. We investigated whether aCL are related to platelet activation, thrombin generation and daily-life ischaemia in patients with chronic coronary artery disease (CAD).

Methods: We measured (medians 25th–75th percentile) IgG, IgM, IgA aCL serum levels (Arbitrary Elisa Units, AEU), prothrombin fragments (F1+2, nmol/l), 24 h urine excretion of 11-dehydrothromboxane B2 (11-DHTXB2, ng/mg creatinine) creatine kinase (CK) and its cardiac isoenzyme CK-MB (IU/l) in 60 patients with angiographically documented CAD and in 40 age and sex matched controls. Patients underwent a 48 h Holter monitoring for assessment of the number and duration of ischaemic episodes.

Results: Patients had higher IgA-aCL levels than controls (3.2 vs 2.4 AEU, $p=0.002$). Increased IgA-ACA levels were related to increased number and duration of ischaemic episodes ($p<0.01$). By ANOVA, patients with ≥ 10 ischaemic episodes (3rd tertile) or duration of ischaemia ≥ 32 min (3rd tertile) had higher IgA-aCL than those with lower ischaemic burden (4.95 vs 3 vs 2.5 AEU, $p=0.002$ and 4.9 vs 3 vs 2.5 AEU, $p=0.001$ respectively). Patients with ≥ 2 ischaemic episodes (2nd and 3rd tertile) had higher 11-DHTXB2, than those with minimal ischaemia ($2<$ episodes, 1st tertile) ($p=0.001$). CK and CK-MB were within normal range after Holter monitoring. Receiver operating curve analysis showed a greater area under the curve for IgA-aCL than for 11-DHTXB2 in predicting severe ischaemia (≥ 10 ischemic episodes or ≥ 32 min duration of ischaemia).

Conclusion: Increasing IgA-aCL levels are associated with increasing ischemic burden in patients with CAD.

While cardiac enzymes are sensitive markers of myocardial necrosis, there are no reliable biomarkers for myocardial ischaemia. Increased production of anticardiolipin antibodies (aCL) has been linked to lipid peroxidation and may cause endothelial dysfunction favouring vasoconstriction.¹ In this study we examined the relationship between aCL, platelet activation and ischaemia during daily-life activities as assessed by 48 h Holter monitoring in chronic coronary artery disease (CAD).

METHODS

We studied 60 patients (53 men, 7 women, age 60 ± 5 years, UK inhabitants) scheduled for elective coronary artery bypass graft (CABG), with effort angina of more than 1 year's duration, documented exercise-induced ischaemia, and over 70% stenosis of more than one coronary artery. Exclusion criteria were evidence of inflammatory diseases, renal/liver failure, heart failure and/or ejection fraction less than 50% or malignant diseases, ECG repolarisation abnormalities, acute coronary events or revascularisation (<6 months) and diabetes. Forty clinically healthy subjects (34 men, 6 women, age 59 ± 13 years) with normal ECG, echocardiogram and treadmill test served as controls.

The study was approved by the hospital's ethics committee and subjects gave their informed consent.

Patients discontinued antiplatelets for 10 days following the current guidelines for CABG. At the end of this period, patients underwent 48 h Holter monitoring (HM) (Marquette 8000 system) to assess the number and duration of ischaemic episodes, blood sampling at 24 h intervals and 24 h urine collections. Anti-anginal therapy was gradually reduced over 48 h to avoid rebound ischaemia and then withheld during the

48 h HM with the exception of sublingual nitrates. All patients were able to complete the study. ST depression >0.1 mV, 60 msec after the J point and lasting >1 minute, was considered to indicate ischaemia.

IgG, IgM and IgA-aCL (arbitrary ELISA units, AEU) were identified by ELISA as previously described.² Urinary 11-DHTXB2 (a marker of platelet activation) was measured by ELISA (Cascade Biochem, Neogen, Reading, UK; sensitivity 0.01 ng/ml) and values were corrected for 24 h urinary excretion of creatinine (ng/mg creatinine). Creatine kinase (CK) and its myocardial isoenzyme (CK-MB IU/l) were measured as indices of myocardial damage.

Statistical analysis

In a pilot study of 12 cases and 8 controls we calculated the means and standard deviations of aCL, PF1+2 and 11-DHTXB2 in each subgroup. We assumed that at least a 30% increase in the levels of the biochemical indices in CAD patients versus normal controls is clinically significant and thus, using a sample size of 40 subjects for the control group, a type (I) error (α) = 0.05 (two-tailed) and a power of 80% for the comparisons between patients and n controls, the sample size for the patients with CAD was calculated to be 60. Assuming a twofold increase in the biomarkers of patients within the upper tertile of the number of ischaemic episodes during HM compared to those within the lowest tertile, the sample was calculated to be 20 per group. Biomarkers are expressed as medians. Differences were analysed by Wilcoxon signed rank or Mann-Whitney U

Abbreviations: aCL, anticardiolipin antibodies; AUC, areas under the curves; CK, creatine kinase; HM, Holter monitoring; ROC, receiver operating characteristic curve

Figure 1 Distribution of IgA anticardiolipin levels in each tertile of ischaemic episodes ($F=7.1$, $p=0.002$). Horizontal lines correspond to median levels of IgA-aCL; p values refer to post-hoc analysis using the Bonferroni correction.

test and correlations by Spearman's rank test. Categorical variables were compared by χ^2 test.

Patients were divided into three groups, based on the tertiles (T1–T3) of the number or duration of ischaemic episodes during the HM (T1: ≤ 1 , $n=20$; T2: 2 to 9, $n=20$; T3: ≥ 10 , $n=20$; and T1: ≤ 1 sec, $n=20$; T2: 2 to 31 sec, $n=20$; T3: ≥ 32 sec, $n=20$).

All non-normal variables were transformed into ranks and were then analysed as dependent variables by ANOVA (General linear model, SPSS 11.5) in a multivariable model including age, sex, hyperlipidaemia, hypertension, smoking, family history of CAD, blood glucose, BMI, number of diseased vessels, anti-anginal medication, the tertiles of the number or duration of ischaemic episodes and their interaction terms as independent variables. The Bonferroni correction was used for post-hoc comparisons. A value of $p<0.05$ was considered significant. Receiver operating characteristic curve analysis (ROC) was used to determine the areas under the curves (AUC) of biomarkers for the prediction of the ischaemic burden.

RESULTS

Patients and controls had similar demographics and distribution of antihypertensive treatment (30% vs 28%), while 45% of both groups were active smokers or had ceased smoking 3 months before inclusion. The patients' subgroups, based on the tertiles of ischaemic episodes, had a similar distribution of anti-anginal treatment. CK and CK-MB levels were normal (<195 IU/l and <24 IU/l) during 48 h HM.

Among biomarkers, only IgA-aCL was higher in patients than in controls (3.2 vs 2.4 AEU, $p=0.002$). Patients who had more than two ischaemic episodes (2nd–3rd tertile) had higher 11-DHTXB2 than controls ($p=0.01$). There was no relationship between aCL, 11-DHTXB2, CK or CK-MB.

Only IgA-aCL was related to number ($r=0.51$, $p=0.008$) and duration of ischaemic episodes ($r=0.52$, $p=0.004$). By ANOVA, patients with ≥ 10 episodes (3rd tertile) or ≥ 32 min ischaemia (3rd tertile) had higher IgA-aCL than those with a lower ischaemic burden (4.9 vs 3 vs 2.5 AEU, $F=7.1$, $p=0.002$ and 4.9 vs 3 vs 2.5 AEU, $F=9.8$, $p=0.001$) (fig 1). Patients with two or more episodes of ischaemia had higher

11-DHTXB2 than those who had less than two episodes (1st tertile) (2.9 vs 2.8 vs 1.8 ng/mg creatinine, $F=8.3$, $p=0.001$), even after adjustment for anti-anginal medication.

By ROC, the AUC for IgA-aCL was greater than for 11-DHTXB2 in predicting more than 10 episodes (80%, 95% CI: 66 to 93, $p=0.001$ vs 61%, 95% CI: 52 to 81, $p=0.05$), or ≥ 32 min of ischaemia (75%, 95% CI: 60 to 88, $p=0.004$ vs 58%, 95% CI: 44 to 74, $p=0.3$).

DISCUSSION

Oxidised low-density lipoprotein may generate aCL.¹ Ischaemia-induced oxidative stress, C-reactive protein and cytokines may lead to cell apoptosis in atherosclerotic lesions. Apoptotic cells relocate mitochondrial cardiolipins to their cell surface³ and may thus trigger aCL production.¹ IgA-aCL has been linked to chronic vascular inflammation⁴ and is involved in the onset and outcome of acute coronary syndromes.⁵ These mechanisms may explain the elevated IgA-aCL shown in our study.

We have also demonstrated a relationship between IgA-aCL and the extent of ischaemia in the absence of elevated cardiac enzymes.

Myocardial ischaemia may cause endothelial cell apoptosis leading to exposure of mitochondrial cardiolipin, and may enhance the oxidative stress causing an increase in IgA-aCL. Conversely, increases in circulating IgA-aCL may also facilitate ischaemia by promoting endothelial dysfunction, complement activation and inflammation.¹

The study was not designed to establish whether aCL might be a cause or a consequence of ischaemia or to elucidate the mechanistic insights of the reported correlations. As oxidised-low density lipoprotein may generate aCL, further studies are required to investigate whether the association between IgA-aCL and ischaemia is independent of increases of oxidised-low density lipoprotein.

In conclusion, IgA-aCL is associated with the severity of daily-life ischaemia in chronic CAD. Its utility as a biomarker of ischaemia remains to be determined in large-scale trials.

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