Original Article

Anti-Beta2-Glycoprotein I Antibodies as Risk Factors for Acute Myocardial Infarction

Aline Ranzolin, Jussara Marilú Bohn, Gary L. Norman, Euler Manenti, Luis Carlos Bodanese, Carlos Alberto von Mühlen, Henrique Luiz Staub
Porto Alegre, RS - Brazil

Objective

To determine whether high levels of antibodies against the phospholipid beta2-glycoprotein I (beta2-gpI) cofactor are associated with an increase in the risk of acute myocardial infarction.

Methods

The study comprised 82 patients with acute myocardial infarction and 82 controls, who were assessed in regard to age, sex, race, hypertension, smoking, previous heart disease, history of diabetes mellitus, and hypercholesterolemia. The following antibodies were detected using immunoassay: anticardiolipin and anti-beta2-gpI IgA, IgG, and IgM. Adjusted odds ratios (OR) for risk factors were obtained through logistic regression.

Results

The mean ages of the cases and controls were, respectively, 57.7 and 51.1 years (P=0.003). Men (P=0.005) and the white race predominated in both groups (P=0.798). Of the risk factors, a history of diabetes (OR=5.3; 95% CI: 1.9 to 14.9; P=0.001) and previous heart disease (OR=4.7; 95% CI: 2.0 to 10.7; P<0.001) were the most consistent associations with myocardial infarction. The frequency of anticardiolipin IgG, IgM, and IgA antibodies did not differ between cases and controls (P=1.000). Anti-beta2-gpI IgA antibodies were more frequent in cases than in controls (P=0.054). The adjusted OR for anti-beta2-gpI IgA antibodies was 3.4 (95% CI: 1.3 to 9.1; P=0.015).

Conclusion

Anti-beta2-gpI IgA antibodies, but not anticardiolipin antibodies, seemed to behave as independent risk factors for myocardial infarction, which may represent a link between autoimmunity and atherosclerosis in patients with acute myocardial infarction.

Key words

antiphospholipid antibodies, anti-beta2-glycoprotein I antibodies, acute myocardial infarction

Hospital São Lucas da PUCRS
Mailing address: Henrique Luiz Staub - Reumatologia - Av. Ipiranga, 6690 - S/220 - Cep 90610-000 - Porto Alegre, RS - Brazil
E-mail: henriquestaub@terra.com.br
Received: 5/30/03
Accepted: 1/6/04
English version by Stela Maris Costalonga

Immunological factors may be involved in the etiopathogenesis of atherosclerosis. The role played by antibodies against phospholipids (PL) or against phospholipid cofactors in the atherosclerotic process has not yet been elucidated [1].

Antiphospholipid antibodies, both anticardiolipin or aCL antibodies and lupus anticoagulant, are related to antiphospholipid syndrome, which is characterized by arterial and venous thromboses and gestational morbidity, being currently considered the most common cause of acquired hypercoagulability among young adults[2].

Acute myocardial infarction occurs in 4 to 20% of the patients with antiphospholipid syndrome [3]. In a recent cohort of 1,000 patients with antiphospholipid syndrome, acute myocardial infarction was observed in 2.8% of the cases [4].

The beta2-glycoprotein I (beta2-gpI) phospholipid cofactor is a natural anticoagulant [5]. This cofactor was found in atherosclerotic plaque [6], and induction of atherosclerosis in receptor-LDL deficient mice immunized with beta2-gpI has been reported [7].

Anti-beta2-gpI antibodies were found in the immunoassays of patients with defined antiphospholipid syndrome [8], but also in patients with thromboembolic pulmonary hypertension [9], cerebral infarction [10], and coronary heart disease [11].

The frequency of anticardiolipin and anti-beta2-gpI antibodies, as well as their role in patients with acute myocardial infarction, has been a controversial issue. Our study provides a complete profile of anticardiolipin and anti-beta2-gpI antibodies in patients with acute coronary heart disease, analyzes their frequency in patients with acute myocardial infarction, and raises the possibility that anticardiolipin and anti-beta2-gpI antibodies act as independent risk factors for acute myocardial infarction.

Methods

This case-control study assessed the titers of anticardiolipin and anti-beta2-gpI antibodies in patients with acute myocardial infarction and in controls. Only incident cases were assessed.

The diagnosis of myocardial infarction was established by cardiologists according to previously reported algorithms, such as clinical history, serial electrocardiographic alterations, and laboratory tests confirming myocardial necrosis [12], and yet the cardiologists continued to ignore the results of antibody titers.

The cases were patients older than 16 years with acute myocardial infarction, who were admitted to the hospital within the first 7 days of symptom onset. They were not selected by sex or race. The patient or his legal representative provided written informed consent. Race/ethnicity was determined by self-identification.

The exclusion criteria were as follows: a) infective endocarditis; b) neoplasias (current or past); c) infection by the human immunodeficiency virus or *treponema pallidum*; d) presence of known hereditary causes of thrombosis, such as homocystinuria or mutation of factor V (Leiden); and e) previous diagnosis of antiphospholipid syndrome or another disease of the connective tissue.

The control group comprised patients without acute myocardial infarction admitted to orthopedic wards due to fractures or muscle-ligament disorders. The exclusion criteria were as follows: a) osteonecrosis; b) infections, neoplasias, hereditary disorders, antiphospholipid syndrome, or diseases of the connective tissue.

Historical, demographic, and clinical data were obtained through a review of medical records and interviews with patients and their families. The risk factors for myocardial infarction were as follows: 1) age, sex, race/ethnicity; 2) history of hypertension (diagnosis confirmed when the systolic or diastolic pressures were > 160 or 95 mmHg, respectively, or when the patient was using antihypertensive medication) [12]; 3) smoking, according to the criteria of the British Council for Medical Research; 4) history of heart disease (atrial fibrillation or coronary heart disease, defined as previous myocardial infarction, angina, or revascularization procedure); 5) history of diabetes mellitus, according to the medical history or the use of insulin or an oral antidiabetes drug; 6) hypercholesterolemia, based on total cholesterol > 200 mg/dL, LDL-cholesterol > 130 mg/dL, or total cholesterol/HDL-cholesterol ratio > 5 [13].

Blood samples were centrifuged and frozen within, at most, 2 hours after collection and stored at –70ºC until laboratory testing with ELISA (enzyme-linked immunoabsorbent assays).

ELISA IgG, IgM, and IgA anticardiolipin antibodies (INOVA Quantalite cardiolipin kits, INOVA Diagnostics, Inc., San Diego, USA) were detected according to a previous report. The results for the IgG and IgM isotypes were reported in IgG phospholipid units (GPL) and IgM phospholipid units (MPL), in which 1 unit equals 1 mg/mL of IgG or IgM. Only samples with moderate to high IgG or IgM anticardiolipin antibody levels (above 20 GPL or 20 MPL) were considered positive in our study. Titers of IgA anticardiolipin antibodies were considered positive when above 15 units [14].

IgA, IgG, and IgM anti-beta2-gpI antibodies were measured according to the technique suggested in a previous report (INOVA Quantalite beta2-gpI kits, INOVA Diagnostics, Inc., San Diego, USA). Briefly, 50 μL of purified human beta2-gpI (at the concentration of 10 μg/mL) was coupled to the orifices of polystyrene plaques. Prediluted controls and diluted serum of patients (1/100) were added to certain orifices, allowing any anti-beta2-gpI antibody present to bind to the immobilized antigen. The samples not bound to the antigen were washed out. Human anti-IgG, anti-IgM, or anti-IgA antibodies (100 μL) bound to peroxidase were added to the orifices. A second incubation allowed antihuman antibodies to bind to any antibody of a patient, which had adhered to the plaque. After washing the unbound antihuman antibodies, the remaining enzymatic activity was measured by the addition of a chromogenic substrate. The assay was assessed with spectrophototic measures. The intensity of the color developed by the sample of the patient was compared with that of the controls. The titers were considered positive when above 20 units for IgG, IgM, or IgA anti-beta2-gp antibodies [15].

Odds ratios with 95% confidence interval (95%CI) were calculated through logistic regression adjusted for age, sex, race, history of hypertension, smoking, previous heart disease, history of diabetes, and hypercholesterolemia. All first-degree interactions between known risk factors for acute myocardial infarction and antibody levels were examined. The Hopkins scale for OR [16] was used as follows: OR between 1 and 1.5 was considered trivial; between 1.5 and 3.5 was considered low; between 3.5 and 9.0 was considered moderate; between 9.0 and 32 was considered strong; and above 32 was considered very strong. The Wald test [17] was used for assessing the significance of OR adjusted for logistic regression. The Fisher exact and chi-square tests were used for comparing categorical variables, and the Student *t* test was used for comparing continuous variables. The significance level of 5% ($P < 0.05$) was adopted. All analyses were obtained by using SPSS for Windows, version 8.0, Chicago, IL.

Results

Our study comprised 82 patients with acute myocardial infarction and 82 controls. The clinical and demographic characteristics of the cases and controls are shown in table I. Most patients with acute myocardial infarction were men and old (P=0.003) (P=0.005), which determined a low risk (OR 2.5; 95% CI: 1.3 to 4.7). The white race predominated among the cases and controls.

The information on the risk factors for cases and controls are shown in table II, and the known risk factors for acute myocardial infarction were more frequent in cases than in controls. A history of diabetes (OR 5.3; 95%CI: 1.9 to 14.9; P=0.001) and previous heart disease (OR 4.7; 95%CI: 2.0 to 10.7; P<0.001) were the 2 most consistent associations with acute myocardial infarction.

Table III categorizes the cases and controls according to the levels of anticardiolipin and anti-beta2-gpI antibodies. The frequency of anti-beta2-gpI IgA, but not of other antibodies, was greater among cases than among controls (P = 0.054).

The adjusted OR for risk factors (age, sex, race, history of hypertension, smoking, previous heart disease, history of DM, and hypercholesterolemia) are shown in table IV [18,19].

The positive test for the anti-beta2-gpI IgG antibody provided an OR of 0.1 (95%CI zero to 1.0); the adjusted P value in the Wald test was borderline for a protective role for this antibody (P=0.055). The occurrence of anti-beta2-gpI IgA antibody determined a moderate risk for acute myocardial infarction (adjusted OR 3.4; 95%CI: 1.3 to 9.1; P=0.015).

Discussion

This case-control study of incident cases included a complete profile of anticardiolipin and anti-beta2-gpI antibodies in patients randomly chosen among adults with acute myocardial infarction.

The mean age of the cases differed significantly from that of controls, and men predominated. It is worth noting that age and sex, as well as other risk factors, were adjusted for logistic regression. Of the known risk factors, a history of diabetes and previous heart disease were the most consistent associations with acute myocardial infarction.

Our results indicate a null frequency of anticardiolipin IgG antibodies in cases of myocardial infarction. The nonadjusted OR (0.3) suggests a protective role for that isotype, but this is only a

Table - Demographic and clinical characteristics of patients with acute myocardial infarction and controls

	Cases (n=82)	Controls (n=82)	P	OR (95%CI)#
Mean age (SD†)	57.7 (10,4)	51.1 (17)	0.003‡	
Men	55 (67.1%)	37 (45.1%)	0.005§	2.5 (1.3-4.7)
White race	74 (90.2%)	73 (89%)	0.798**	1.1 (0.4-3.1)

* Odds ratio with 95% confidence interval; † SD - standard deviation; ‡ Student *t* test; § chi-square test.

Table II - Profile of the risk factors in cases and controls

	Cases (n=82)	Controls (n=82)	P*	OR (95%CI)†
Risk factors				
History of hypertension	46 (56.1%)	22 (26.8%)	<0.001	3.5 (1.8-6.7)
Smoking	46 (56.1%)	28 (34.1%)	0.005	2.5 (1.3-4.6)
Previous heart disease	30 (36.6%)	9 (11%)	<0.001	4.7 (2.0-10.7)
History of diabetes mellitus	21 (25.6%)	5 (6.1%)	0.001	5.3 (1.9-14.9)
Hypercholesterolemia	34 (41.5%)	16 (19.5%)	0.002	2.9 (1.4-5.9)

* Chi-square; † odds ratio with 95% confidence interval.

Table III - Frequency of anticardiolipin and anti-beta2-gpI antibodies for cases and controls

	Cases (n=82)	Controls (n=82)	P
aCL IgG positive	0	1 (1.2%)	1.000*
aCL IgM positive	4 (4.9%)	3 (3.7%)	1.000*
aCL IgA positive	1 (1.2%)	0	1.000*
anti-beta2-gpI IgG positive	2 (2.4%)	4 (4.9%)	0.682*
anti-beta2-gpI IgM positive	12 (14.6%)	8 (9.8%)	0.340**
anti-beta2-gpI IgA positive	22 (26.8%)	12 (14.6%)	0.054**

* Fisher exact test; † chi-square test.

Table IV - Odds ratio for anticardiolipin and anti-beta2-gpI antibodies adjusted for risk factors

	OR#	IC95%†	P‡
aCL IgG§	noncalculated	---	---
aCL IgM	2.0	0.2-21.7	0.570
aCL IgA§	noncalculated	---	---
anti-beta2-gpI IgG	0.1	0-1.0	0.055
anti-beta2-gpI IgM	1.2	0.4-4.0	0.726
anti-beta2-gpI IgA	3.4	1.3-9.1	0.015

*OR - adjusted odds ratio for demographic data and risk factors; †95%CI - 95% confidence interval; ‡Wald test [17]; § noncalculated logistic regression due to null frequency in cases or controls. The nonadjusted OR after Agresti correction [18] was 0.3 for aCL IgG test and 3.0 for aCL IgA.

hypothesis (P = 1.000). Our group has already reported a very low prevalence (1.2%) of anticardiolipin IgG in acute myocardial infarction [19]. However, the presence of anticardiolipin IgG has been linked to risk, although low, of infarction according to a previous report [20]. Two previous cohorts have reported a time-dependent association of anticardiolipin IgG antibodies with acute myocardial infarction [21,22].

Our data regarding the anticardiolipin IgM isotype were not compatible with the association with acute myocardial infarction. The prevalence of anticardiolipin IgM in our study in 1993 was null [19]. A recent study [23] associating IgM anticardiolipin and stroke triggers the discussion of the role played by infections in the anti-phospholipid IgM response.

The frequency of anticardiolipin IgA in our cases of acute myocardial infarction was very low (1.2%). No control was positive. The nonadjusted OR of 3.0 may suggest an association with ischemic outcome. However, the nonadjusted P value of 1.000 makes this hypothesis unlikely. A prospective association of the anticardiolipin IgA isotype with acute myocardial infarction has been previously reported [21]. Therefore, the aCL IgA isotype, whose immunoassay has not yet been internationally standardized, should be studied in these patients.

The relation between beta2-gp I and atherosclerosis is intriguing. Atheromas contain beta2-gpI [6]. Our study raises the possibility that anti-beta2-gpI antibodies may be associated with the risk of acute myocardial infarction.

In our study, the frequency of anti-beta2-gpI IgG antibodies was lower in cases than in controls. The low adjusted OR (0.1) and the adjusted P value of 0.055 point towards the possibility of a protective role of that antibody.

Farsi et al [11] reported an association of anti-beta2-gpI IgG antibodies with coronary atherosclerosis (particularly unstable angina). However, data from the Honolulu Heart Program [20] point towards an insignificant frequency of anti-beta2-gpI IgG antibodies as compared with that of the controls. Two other studies have also ruled out the possibility of anti-beta2-gpI IgG antibodies being linked to coronary heart disease [3,24]. In addition, the presence of anti-beta2-gpI IgG, as well as of anticardiolipin antibodies, does not seem to be a risk for coronary restenosis after angioplasty [25].

In our study, the anti-beta2-gpI IgM isotype showed no association with acute myocardial infarction. Theoretically, an occasional anti-beta2-gpI IgM response observed in myocardial infarction could result from infection or previous tissue necrosis.

Significant titers of anti-beta2-gpI IgA antibodies were detected in patients with acute myocardial infarction as compared with those in controls. The OR and the adjusted P value indicate that a positive test for anti-beta2-gpI IgA behaves as an independent risk factor for acute myocardial infarction. Likewise, an association between this antibody and the risk of cerebral infarction has been recently reported by our group [26].

The association of anti-beta2-gpI IgA antibodies with acute

myocardial infarction is controversial. The great majority of our patients with anti-beta2-gpI IgA antibodies are aCL IgA-negative. As previously suggested, anti-beta2-gpI IgA and aCL IgA may comprise AAF of different specificities [27].

Whether patients with acute myocardial infarction, who are anti-beta2-gpI IgA positive, but have a negative anticardiolipin IgA test, should be managed as having antiphospholipid syndrome is still controversial. The 1999 international consensus for the diagnosis of antiphospholipid syndrome does not include anti-beta2-gpI antibodies [2]. The incorporation of these antibodies into the criteria of antiphospholipid syndrome has been recently proposed [28].

It is worth noting that antibodies against the prothrombin phospholipid cofactor have also been implicated as risk factors for acute myocardial infarction in middle-aged men according to a report [29]. Low anexin V levels, a phospholipid cofactor with anticoagulant properties, have been recently reported in patients with a history of early acute myocardial infarction [30].

In conclusion, anti-beta2-gpI IgA antibodies seemed to behave as independent risk factors for acute myocardial infarction in our study. The need for testing anti-beta2-gpI antibodies, particularly IgA, in patients with coronary heart disease should be discussed and their predictive value assessed.

Although beta2-gpI is found in atherosclerotic plaque [6], a pathogenic role for anti-beta2-gpI IgA antibodies in acute myocardial infarction has not yet been confirmed. Epiphenomenon or not, the occurrence of these antibodies in acute myocardial infarction may represent 1 of the links between autoimmunity and coronary atherosclerosis. The clinical implications of such findings may be clarified in the near future.

Acknowledgements

We thank Vicki J. Nelson for technical support, and the Fundos Remanescentes da Sociedade Brasileira de Reumatologia.

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