Autoantibodies to the Atheroma Component Beta2-Glycoprotein I and Risk of Symptomatic Peripheral Artery Disease

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Autoantibodies to the Atheroma Component Beta2-Glycoprotein I and Risk of Symptomatic Peripheral Artery Disease

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Peripheral artery disease (PAD) is mostly related to atherosclerosis. Autoimmunity and, in particular, antibodies to cardiolipin (aCL) and phospholipid cofactors such as beta2-glycoprotein I (beta2-gpI) might influence the development of atheroma. Beta2-glycoprotein I (beta2-gpI) has been found in atheroma. It has previously been shown that immunoglobulin A (IgA) anti-beta2-gpI antibodies are associated with a risk of cerebral ischemia and myocardial infarction. This case control study aimed to determine whether elevated levels of aCL/anti-beta2-gpI antibodies are associated with a risk of symptomatic PAD (sPAD). Cases comprised a nonselected population of patients with sPAD (intermittent claudication or critical ischemia). Patient recruitment was based on arteriography changes. Controls were selected from patients admitted to orthopedic wards as a result of fractures or muscle-ligamentous disorders. Age, sex, race, hypertension, smoking, diabetes mellitus, and hypercholesterolemia were evaluated as risk factors in both groups. IgG/IgM/IgA aCL and anti-beta2-gpI were detected by enzyme-linked immunoabsorbant assays (ELISA). To estimate the grade of association of antibodies with sPAD, odds ratios (OR) were calculated. Logistic regression was utilized for adjustment of confounding factors. Seventy-seven cases and 93 controls were studied. The mean age was 61.5 years for cases and 47.5 years for controls ($p < 0.001$). Among the risk factors evaluated, the presence of hypertension showed the strongest association with sPAD (OR 12.1; 95%CI 5.8–30). The presence of IgA anti-beta2-gpI was independently associated with sPAD (OR 5.4; 95%CI 1.8–15.8; $p = 0.01$). IgA aCL was strongly associated with the outcome (nonadjusted OR 11.5 after Agresti correction). IgA aCL and IgA anti-beta2-gpI antibodies were not associated with any known risk factors for sPAD or with arteriography changes. The occurrence of these autoantibodies might represent one of the links between autoimmunity and atherosclerosis in patients with sPAD.

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Introduction

Peripheral artery disease (PAD) is related to atherosclerosis. Immunologic factors are potentially involved in atherogenesis.[1] The phospholipid cofactor beta2-glycoprotein I (beta2-gpI) is an atherosclerotic plaque component.[2] Therefore, an immunologic response to beta2-gpI might influence atheroma progression in patients with PAD.

Anticardiolipin (aCL) antibodies have been linked to the so-called antiphospholipid syndrome (APS). The APS is currently considered the most frequently acquired thrombophilia of young adults.[3] Nevertheless, thrombosis of peripheral arteries was not a common feature of APS, according to a large European cohort.[4] A few studies of patients with PAD have looked at the whole profile of aCL antibodies, including the immunoglobulin A (IgA) isotype.

Beta2-gpI, an approximately 50 kilodalton natural anticoagulant, is a target for aCL antibodies.[5] Immunization of mice with beta2-gpI generates anti-beta2-gpI antibodies, aCL antibodies, and APS.[6] A previous report has claimed that anti-beta2-gpI antibodies are more specific than the aCL assay for detection of thrombosis in patients with systemic lupus erythematosus (SLE).[7] Apart from this, the anti-beta2-gpI assay has not been included in the APS criteria to date.[3] There is a growing interest in the role of these autoantibodies in the atherogenic process.

Our group recently reported a risk association of IgA anti-beta2-gpI antibodies with cerebral ischemia[8] and myocardial infarction.[9] There has been little information on anti-beta2-gpI antibodies in patients with PAD. In this study, we set out to analyze the frequency of immunoglobulin G/immunoglobulin M/immunoglobulin A (IgG/IgM/IgA) aCL and anti-beta2-gpI antibodies in patients with symptomatic PAD (sPAD). We also address the possibility that these antibodies work as independent risk factors for sPAD.

Methods

Patients

We assessed aCL and anti-beta2-gpI antibodies in patients with sPAD and in controls enrolled in a case-control study. Only incident cases were evaluated. Diagnosis of sPAD was established by a vascular surgeon or physician according to a previously reported algorithm. Subsequently, sPAD was confirmed by: (1) presence of intermittent claudication for at least 3 months or critical ischemia (rest pain, ulcers, or gangrene of lower limbs); (2) arteriographic abnormalities including arterial wall lesion, stenosis, or arterial occlusion.[10]

All case patients were at least 18 years old. Patients were not selected by sex or race. Either the patient or a proxy gave informed consent. Self-identification determined race/ethnicity.

Exclusion criteria for cases were as follows: (1) infective endocarditis, (2) neoplasms (current or past), (3) infection by the human immunodeficiency virus or *Treponema pallidum,* (4) presence of known heritable causes of thrombosis such as homocystinuria or factor V (Leiden) mutation, (5) previous diagnosis of APS or other connective tissue disorder (CTD).

The control group was recruited from patients without sPAD admitted in the Orthopaedic Infirmary for fractures or muscule-ligamentous disorders. Exclusion criteria were the following: (1) osteonecrosis; (2) cardiac abnormalities, infections, neoplasms, heritable diseases, APS, or CTD as listed above for "cases."

Historical, demographic, and clinical information was obtained from chart review and interview with patients and family. Risk factors for PAD included in our clinical protocol were the following: (1) age, sex, race/ethnicity; (2) history of hypertension[11]; (3) current smoking; (4) history of diabetes mellitus (DM); (5) hypercholesterolemia.[12]

Specimens

Serum specimens were centrifuged and frozen within 2 hours of collection and stored at –70°C until the aPL enzyme-linked immunoabsorbant assays (ELISA) were performed with standardized kits.

The IgG/IgM/IgA aCL antibody ELISA (INOVA QUANTA Lite™ cardiolipin kits, INOVA Diagnostics, Inc., San Diego, CA, USA) were performed according to a previous description. IgG and IgM isotype results were reported as IgG phospholipid units (GPL) and IgM phospholipid units (MPL), whereby 1 unit is equal to 1 μg/mL of IgG or IgM. Only samples with moderate or high levels of IgG or IgM aCL antibodies (above 20 GPL or 20 MPL) were considered as positive in our study. IgA aCL antibody titers were considered positive when above 15 units.[13]

The IgG, IgM, and IgA anti-beta2-gpI antibodies ELISA were performed as previously described

(INOVA QUANTA Lite™ beta2-gpI kits, INOVA Diagnostics, Inc., San Diego, CA, USA). In brief, 50 µL of purified human beta2-gpI (concentration 10 µg/mL) was bound to the wells of a polystyrene microwell plate. Prediluted controls and diluted patient sera (1/100) were added to separate wells, allowing any anti-beta2-gpI antibodies present to bind to the immobilized antigen. Unbound samples were washed away, and an enzyme labeled anti-IgG, anti-IgM, or anti-IgA (100 µL) was added to each well. A second incubation allowed the enzyme-labeled antihuman antibodies to bind to any patient antibodies that had become attached to the microwells. After any unbound enzyme-labeled antihuman antibodies were washed away, the remaining enzyme activity was measured by adding a chromogenic substrate and measuring the intensity of the color that developed. The assay was evaluated by spectrophotometrically measuring and comparing the color intensity that developed in the patient wells with the color in the control wells. Titers were considered positive when above 20 units for IgG and IgM anti-beta2-gpI antibodies and, arbitrarily, when above 25 units for IgA anti-beta2-gpI.[14]

Data Analysis

Odds ratios (OR) with 95% confidence intervals (95%CI) were calculated by logistic regression analysis, with adjustment for age, sex, race, hypertension, current smoking, diabetes mellitus, and hypercholesterolemia. All first-order interactions between historical PAD risk factors and aCL/anti-beta2-gpI status were examined. The Hopkins scale for OR[15] was utilized, whereby an OR between 1–1.5 was considered as trivial; between 1.5–3.5 as small; between 3.5–9.0 as moderate; between 9.0–32 as strong; and above 32 as very strong. Fisher's exact test and chi-square analysis were used for comparison of categorical variables, and the Student's t test was used for comparison of continuous variables; a level of 5% ($p < 0.05$) was considered significant. All analyses used procedures of the SPSS for Windows, version 11.5, Chicago, IL.

Results

There were 77 patients with sPAD and 93 controls included in our study. Owing to the inclusion criteria, all cases presented arteriographic abnormalities.

The demographic and clinical characteristics of cases and controls are shown in Table I. Patients with sPAD were more likely to be older ($p < 0.001$). Cases and controls did not differ regarding sex and race. In both groups, male (weakly) and white ethnicity (largely) predominated.

Risk factor information for cases and controls are seen in Table II. All risk factors were significantly more frequent in cases than controls. History of hypertension (OR 12.1) yielded the strongest association with sPAD. History of DM (OR 5.4) and current smoking (OR 3.9) associated moderately with the outcome, whereas hypercholesterolemia associated weakly with sPAD (OR 2.0). The occurrence of more than 1 risk factor was significantly seen in cases as compared to controls.

Table III categorizes cases and controls by aCL/anti-beta2-gpI antibodies status. The frequency of IgG or IgM aCL, as well as of IgG or

Table I. Demographic and clinical characteristics of sPAD patients and controls.

	Cases (n = 77)	Controls (n = 93)	p	OR (95%CI)‡
Mean age, years (SD)	61.5 (10.3)	47.5 (18.8)	<0.001*	
Males	45 (58%)	47 (50.5%)	0.28†	1.4 (0.8–26)
Black race	14 (18%)	10 (10.8%)	0.25†	1.8 (0.7–4.8)

*Student's t test.

†Chi-square test.

‡Odds ratio with 95% confidence interval.

Table II. Risk factors profile in cases and controls.

Risk Factors	Cases (n = 77)	Controls (n = 93)	p*	OR (95%CI)†
History of hypertension	60 (78%)	21 (23%)	<0.001	12.1 (5.8–30)
Current smoking	50 (65%)	30 (32%)	<0.001	3.9 (2–7.3)
History of diabetes mellitus	18 (23%)	5 (5,4%)	<0.001	5.4 (1.9–15.2)
Hypercholesterolemia	25 (32%)	18 (19.4%)	0.05	2 (1–4)
More than 1 risk factor	48 (62.3%)	18 (19.4%)	<0.0001	6.9 (3.2–14.6)

*Chi-square test
†Odds ratio with 95% confidence interval.

Table III. aCL and anti-beta2-gpI antibodies frequency for cases and controls.

	Cases (n = 77)	Controls (n = 93)	p*	OR (95%CI)†
Positive IgG aCL	5 (6.5%)	1 (1.1%)	0.09	6.4 (0.7–55.9)
Positive IgM aCL	5 (6.5%)	3 (3.2%)	0.47	2.1 (0.5–9)
Positive IgA aCL	4 (5.2%)	0	0.04	11.5 (1.1–inf.)‡
Positive IgG anti-beta2-gpI	2 (2.6%)	5 (5.4%)	0.46	0.5 (0.1–2.5)
Positive IgM anti-beta2-gpI	5 (6.5%)	10 (10.8%)	0.42	0.6 (0.2–1.8)
Positive IgA anti-beta2-gpI	30 (39%)	9 (9.7%)	<0.001	6 (2.6–13)

*Fisher's exact test.
†Odds ratio with 95% confidence interval.
‡OR obtained after Agresti correction[16]; 95%CI 1.1–infinite owing to the null frequency in controls.

IgM anti-beta2 antibodies, did not significantly differ in cases and controls. The frequency of IgA aCL antibodies was 5.2% in cases and null in controls (p = 0.04). The nonadjusted OR obtained after Agresti correction[16] was 11.5 (95%CI 1.1–infinite owing to the null frequency in controls). The frequency of IgA anti-beta2-gpI was 39% in cases and 9.7% in controls (p < 0.001). The nonadjusted OR for this antibody was 6 (95%CI 2.6–13).

The OR for aCL/anti-beta2-gpI antibodies adjusted for risk factors (age, sex, race, history of hypertension, current smoking, history of DM, and hypercholesterolemia) are seen in Table IV.

According to the Hopkins scale,[15] the presence of IgG or IgM aCL and of IgG or IgM anti-beta2 antibodies did not confer a significant risk for PAD. Concerning the IgA aCL isotype, logistic regression was not calculated owing to the null frequency in controls. The nonadjusted OR (11.5) was compatible with strong association of IgA aCL with sPAD. The occurrence of IgA anti-beta2-gpI antibodies determined a moderate risk of sPAD (p = 0.01; OR 5.4; 95%CI 1.8–15.8). This association occurred independently of the presence of other risk factors.

By bivariate analysis, cases and controls tested for IgA aCL and IgA anti-beta2-gpI were evaluated as the association, if any, with known risk factors for PAD (age, sex, race, history of hypertension, current smoking, history of DM, and hypercholesterolemia) and with arteriography

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

	Cases (n = 77)	Controls (n = 93)	p	OR (95%CI)†
Positive IgG aCL	5 (6.5%)	1 (1.1%)	0.22	4.6 (0.4–49.3)
Positive IgM aCL	5 (6.5%)	3 (3.2%)	0.15	4.5 (0.6–33.7)
Positive IgA aCL*	4 (5.2%)	0	–	–
Positive IgG anti-beta2-gpI	2 (2.6%)	5 (5.4%)	0.46	0.5 (0.1–3.6)
Positive IgM anti-beta2-gpI	5 (6.5%)	10 (10.8%)	0.53	0.6 (0.1–3)
Positive IgA anti-beta2-gpI	30 (39%)	9 (9.7%)	0.01	5.4 (1.8–15.8)

*A p value and adjusted OR not calculated owing to the null frequency in controls. OR after Agresti correction[16] was 11.5 (95%CI 1.1–infinite).

†Odds ratio with 95% confidence interval adjusted for age, sex, race, history of hypertension, current smoking, history of diabetes mellitus, and hypercholesterolemia.

changes (arterial wall lesion, stenosis, occlusion). No associations were found ($p > 0.05$).

Discussion

The role of aCL/anti-beta2-gpI antibodies in the atherogenic process of patients with PAD is still unclear. This case-control study of incident cases includes a complete profile of aCL and anti-beta2-gpI antibodies in patients with sPAD.

We studied an unselected population of adults with sPAD. The control group comprised an orthopedic group of patients with no symptoms of PAD. Mean age in cases (61.5 years) differed significantly from the controls (45.7 years), but age, as a risk factor, was adjusted by logistic regression. Caucasoids (strongly) and males (weakly) predominated in both cases and controls. All known risk factors were more frequent in cases, but hypertension yielded the strongest association with PAD in our study. A recent report[17] accounted for a greater relevance of smoking and hypertension as compared to hypercholesterolemia and DM as risk factors for cardiovascular disease (including PAD) in primary care. In another study, current smoking and history of DM, but not hypercholesterolemia, were independently associated with greater PAD incidence.[18] As seen, the frequency of traditional risk factors for PAD differs in recent reports.[17,18] In our study, the presence of more than 1 risk factor was significantly greater in cases than in controls.

The IgA aCL antibody determined a strong risk of association with sPAD in our study. Given the null frequency of this isotype in the control group, logistic regression was not calculated. Nevertheless, the high nonadjusted OR (11.5) was compatible with a consistent association with the outcome.

Currently there is no consensus on the recommended use and interpretation of IgA aCL testing. The Sapporo consensus for APS classification[3] did not include this isotype. In practical terms, IgA aCL can be the sole antiphospholipid antibody (APA) in some patients with APS.[13] In animal models, this antibody is as thrombogenic as the other aCL isotypes.[19]

In patients with atherosclerotic disease, IgA aCL has not been associated with risk of cerebral ischemia[8,20] or myocardial infarction.[9] Concerning peripheral disease, Lam et al[21] related that elevated IgG/IgM/IgA aCL are independent risk factors for PAD progression in patients previously submitted for surgery of lower limbs. Thus, the role of IgA aCL in patients with atherosclerosis has yet to be clarified.

The frequency of IgG or IgM aCL in our patients with sPAD did not significantly differ from that in controls. Also, these aCL isotypes did not yield any risk of sPAD. In contrast, IgG and IgM aCL associated with risk of intermittent claudication in a 40-patient survey. Lower cutoff (10 GPL, 7 MPL) were utilized as compared to our study.[22]

In patients with intermittent claudication and thrombophilia, IgM aCL antibodies comprised the most frequent abnormality.[23] Puisieux et al[24] found a significant frequency (16%) of IgG aCL in patients with PAD (cutoff 15 GPL). The antibody was linked to both global and cardiovascular mortality. Similarly, Friehs et al[25] reported a high prevalence of IgG aCL in patients with cerebral and peripheral ischemia. The different cutoff for IgG/IgM aCL utilized in various studies[22,24] might explain these discrepant results in PAD.

Beta2-glycoprotein I appears to be an immunogenic component of atheroma. Immunization of LDL-receptor-deficient mice with beta2-gpI triggered atherosclerotic lesions.[26] Oral administration of human beta2-gpI in mice prevented atheroma formation by blockage of cell response and activation of interleukin-4 (IL-4) and IL-10, antiatherogenic cytokines.[27] Therefore, the humoral response to beta2-gpI in atherosclerotic patients is of current interest.

Our data regarding IgG and IgM anti-beta2-gpI antibodies revealed absence of association with sPAD. This finding is concordant with our previous results in cerebral ischemia[8] and myocardial infarction.[9] However, increased levels of anti-beta2-gpI antibodies, particularly IgA, have been reported in young patients with acute coronary syndrome (ACS).[28] According to Lopez et al,[29] the presence of IgG/IgM/IgA anti-beta2-gpl was highly predictive for arterial thrombosis in patients with APS.

IgA anti-beta2-gpI antibodies have not been studied in peripheral disease. This isotype was definitely more frequent in our patients with sPAD as compared to controls. Moreover, IgA anti-beta2-gpI antibodies independently associated with risk of sPAD (adjusted OR 5.4; 95%CI 1.8–15.8; $p = 0.01$). This finding corroborates our data on cerebral ischemia[8] and myocardial infarction.[9] The antibody related neither to known risk factors for PAD nor to arteriography changes.

Our recent data on IgA anti-beta2-gpI antibodies suggest that this isotype behaves as a uniform and reproducible risk marker for various anatomic sites of atherosclerosis. Risk estimators were higher in PAD as compared to cerebral ischemia and myocardial infarction.[8,9] The more extensive atherosclerotic disease and consequent higher antigenic exposition in PAD might account for these differences.

The immunogenicity of beta2-gpI and oxidized low-density lipoprotein (oxLDL) at the atheroma has been well recognized.[30] A recent study in this hospital claimed that antibodies to oxLDL are elevated in ACS as compared to chronic patients with coronary disease. These antibodies might relate to plaque instability.[31]

As a complex, beta2-gpI/oxLDL can block the interaction or oxLDL with scavenger receptors at the macrophage surface. IgG antibodies directed to the complex can bind Fc gamma receptors of macrophages, leading to internalization of oxLDL and foamy cell appearence.[30,32]

Of recent interest, IgG antibodies to the beta2-gpI/oxLDL complex seem to associate with arterial thrombosis in patients with APS.[33] In another study, the IgM anti-beta2/gpI-oxLDL isotype was particularly frequent in cases of rheumatoid arthritis.[34] As a whole, these antibodies, probably a distinct subtype of APA, appear to correlate with early atherosclerosis in patients with APS or CTD. Of note, the IgA isotype anti-beta2-gpI/oxLDL was not tested in these studies.[30,32-34]

Whether IgA anti-beta2-gpI antibodies from patients with PAD bind solely to the glycoprotein or to the beta2-gpI/oxLDL complex is yet to be resolved. Of interest, IgA alpha receptors have been identified in macrophages. IgA receptor signaling on macrophages was found to trigger phagocytosis and release of inflammatory mediators.[35,36] Supposing that IgA anti-beta2-gpI antibodies bind the complex, occupation of alpha receptors at the macrophage could generate foamy cells. This hypothetical model might be an explanation for the consistent link of IgA anti-beta2-gpI antibodies with atherosclerotic disease[8,9] (and this study).

Other questions arise from our findings on PAD. Are the IgA aCL and IgA anti-beta2-gpI related antibodies in this circumstance? Should these patients be managed as having APS? Nonetheless, we cannot rule out the possibility that these IgA APA isotypes behave as an epiphenomenon in patients with atherosclerosis

In summary, we report a risk association of IgA aCL with sPAD. IgA anti-beta2-gpI antibodies worked as independent risk factors for sPAD in our patients. The occurrence of these autoantibodies might be representative of a link between autoimmunity and atheromatosis of the peripheral circulation.

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