Antiphospholipid and Antinuclear Antibodies in Young Patients after Myocardial Revascularization Procedures

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ABSTRACT:
Background: The role of autoimmune factors in the etiology of coronary artery disease (CAD) was suggested in numerous studies but has not been definitively determined.
Objectives: To assess the possible influence of antiphospholipid and antinuclear antibodies on atherosclerosis development in young patients after myocardial revascularization procedures.
Methods: The study group included 39 patients younger than 45 years with CAD who underwent myocardial revascularization. Serum levels of antiphospholipid (aPL), antinuclear (ANA) and antineutrophil cytoplasmatic (ANCA) antibodies were tested within 1 month after the procedure.
Results: All three types of aPL were significantly higher in CAD patients when compared to healthy controls: anti-β2-glycoprotein I (aβ2GPI), both immunoglobulin (Ig)G and IgM classes (median 4.10 SGU, range 3.45–21.63 vs. 0.76, 0.12–6.01, $P < 0.001$, and 2.82 SGU, 1.44–11.70 vs. 1.08, 0.44–3.64, $P < 0.001$, respectively); anticardiolipin antibodies (aCL) both IgG and IgM classes (3.13 GPL, 1.32–14.03 vs. 2.42, 0.96–18.45, $P = 0.0037$, and 6.94 MPL, 1.90–26.40 vs. 4.32, 1.9–28.73, $P < 0.008$, respectively); and lupus anticoagulant (LA) (27.7% vs. 0%, $P = 0.005$). ANA were elevated in one patient and ANCA in 23 (60%). The levels of aPL did not correlate with the presence of a clot in a coronary vessel detected during angiography or with exacerbation of coronary artery atherosclerosis.
Conclusions: In young patients with CAD who underwent myocardial revascularization the levels of aPL were significantly higher than in young healthy subjects. Thus, besides the classic risk factors for CAD, autoimmunity may play an important role in atherosclerotic plaque formation and progression.

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KEY WORDS: coronary artery disease (CAD), antiphospholipid antibodies (aPL), atherosclerosis, autoimmunity

C onventional risk factors such as diabetes, arterial hypertension, nicotinism, sedentary lifestyle and dyslipidemia are well-proven causes of atherosclerosis and coronary artery disease (CAD). These factors enable the detection of individuals at risk for CAD. Controlling the extent of these factors and modifying them may limit cardiovascular risk. However, there is

a group of patients for whom this evaluation is not effectual [1]. Several inflammatory and immune factors have been shown to markedly contribute to the development of atherosclerosis [2,3]. Among the most frequently tested new risk factors, significance was confirmed for high sensitive C-reactive protein (hsCRP), homocysteine and oxidized low density lipoprotein (oxLDL) [2]. The role of autoimmune factors was tested in numerous studies, but their role remains unclear.

In autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), antiphospholipid antibodies (aPL) are the main cause of organ dysfunction and may play a role in atherosclerosis [4]. Antibodies such as rheumatoid factor (RF) and antinuclear antibodies (ANA) are also strong predictors of cardiovascular events [3,5]. Lupus anticoagulant (LA) in SLE patients is associated with a high incidence of myocardial infarction (MI) [6].

Some authors have demonstrated that anticardiolipin antibodies (aCL), anti-β2-glycoprotein I (aβ2GPI) and LA may play a role in the development of atherosclerosis in patients without autoimmune disease [7], but other studies did not confirm this finding [8]. In animal models, aβ2GPI has exhibited pro-atherogenic activity [9] and increased myocardial infarct size [10]. In clinical trials, aβ2GPI was associated with more severe CAD, higher risk of further revascularization, and stent thrombosis [11]. Furthermore, LA and aCL have been shown to correlate with the incidence of MI [7], but their role in generating atherosclerosis is controversial [8].

The data obtained from single published studies performed in young patients under the age of 45 seem to support the results noted above [7,11,12]. However, no prospective studies have examined autoimmunity in young patients diagnosed with CAD after myocardial revascularization procedures. We therefore undertook to assess the possible role of antiphospholipid and antinuclear antibodies in the development of atherosclerosis in young patients after myocardial revascularization procedures.

PATIENTS AND METHODS

The study was conducted in a group of 39 patients under the age of 45 years with coronary artery disease who under-

Table 1. General characteristics of the study and control groups

	Study group (n=39)	Control group (n=41)
Age, mean ± SD	39.41 ± 4.68	39.26 ± 3.94
Male sex (%)	33 (84.6)	7 (170)
Conventional risk factors		
Hypercholesterolemia (%)	34 (87.1)	19 (46.3)
Nicotinism (%)	29 (74.4)	9 (21.9)
Obesity (%)	10 (25.6)	5 (12.2)
Arterial hypertension (%)	24 (61.5)	10 (24.4)
Diabetes mellitus type 2 (%)	4 (10.2)	2 (4.9)
First manifestation of CAD		
Age at first manifestation, mean ± SD	38.64 ± 5.12	–
Acute myocardial infarction (%)	31 (79.4)	–
ST-elevation myocardial infarction (%)	19 (48.7)	–
Stable CAD (%)	7 (17.9)	–
History of PCI (%)	34 (87.1)	–
History of CABG (%)	4 (10.7)	–
Optimal medical therapy (%)	3 (7.7)	–

CAD = coronary artery disease, PCI = percutaneous coronary intervention, CABG = coronary artery bypass graft

Table 2. Study group characteristics according to type of revascularization and type of angiographic lesions

	PCI (n=32)	CABG (n=4)
Age, mean ± SD	39.54 ± 4.63	38.25 ± 5.74
Male sex (%)	27 (84)	3 (75)
More than one vessel affected (%)	9 (28)	2 (50)
LAD affected (%)	16 (50)	3 (75)
LM affected (%)	2 (6)	3 (75)
Thrombus (%)	8 (25)	0 (0)
Three or more conventional risk factors (%)	18 (56)	4 (100)

PCI = percutaneous coronary intervention, CABG = coronary artery bypass graft, LAD = left anterior descending artery, LM = left main artery

and EY2C9 for IgM). Values exceeding the 99th percentile of a healthy population (both aCL and aβ2GPI) were regarded as positive. In our study, cutoff values were aCL IgG 10 GPL, aCL IgM 20 MPL, aβ2GPI IgG 20 SGU, and aβ2GPI IgM 20 SGU.

Lupus anticoagulant (LA) detection was performed on the basis of a three-step analysis recommended by the International Society on Thrombosis and Haemostasis. Antinuclear antibodies (ANA) were assessed by indirect fluorescence (Hep-2 cells, Euroimmun GmbH, Lubeck, Germany). The type of ANA was identified by immunoblotting (Euroline System, Euroimmun GmbH, Lubeck, Germany). Titers > 1:160 were regarded as positive for ANA, while titers > 1:10 were considered positive for ANCA [13].

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistica Ten Sigma software. Continuous variables were compared using the Mann-Whitney U test. Differences in proportions were evaluated with the chi-square test. The level of statistical significance was determined at $P < 0.05$. All numerical data were presented as mean ± standard deviation, median and range, or as proportions.

RESULTS

The levels of all types of aPL were significantly higher in CAD patients: aβ2GPI in both IgG and IgM, and aCL in both IgG and IgM; LA was also more frequent in the examined patients [Table 3]. Higher levels of serum aPL were detected in males than in females, but this difference was not significant (fewer women had CAD than men) [Table 4].

In the group of investigated patients, 12 (30.7%) had at least one elevated aPL measurement; all of these patients were males, and among them 3 were double positive (7.7%). The levels of aPL did not correlate with the presence of a thrombus in a coronary vessel detected during angiography. The patients with double or triple vessel disease did not differ from patients with single vessel disease in terms of aPL levels.

went myocardial revascularization at John Paul II Hospital, Krakow, Poland. Informed consent was obtained from each patient and the study was approved by the Ethical Committee of the Jagiellonian University in Krakow. Exclusion criteria were the presence of previously diagnosed autoimmune diseases, concomitant treatment with immunosuppressive drugs, any thyroid disorder, chronic infections and known malignancies.

The clinical and laboratory features of the group are shown in Table 1. Clinical manifestations of CAD in the patients were diverse, the most common initial presentation being acute MI, among which the most frequent was ST elevated MI (STEMI) (19 patients, 48.7%). Thirty-four patients (87.1%) were treated percutaneously [Table 2]. In four cases coronary artery bypass graft was necessary, and in three patients admitted with STEMI after thrombus washout by the catheter the target vessel remained unaffected by atherosclerosis and stent implantation was not necessary. Blood samples were obtained within 1 month after the procedure.

The control group comprised 41 healthy volunteers: 7 males and 34 females. None of the participants in the control group had a history or symptoms of CAD. Risk factors were less frequent in the control group than in CAD patients; however, their prevalence was quite similar to that observed in the general population [Table 1].

LABORATORY TESTS

aCL and aβ2GPI antibodies were measured with home-made immunoenzymatic assays (ELISA). For both types of auto-antibodies, IgG and IgM antibody classes were detected. To measure aβ2GPI, the Sapporo Standard was used (murine monoclonal antibodies against human β2GPI-HCAL for IgG

Table 3. Laboratory tests in the two groups

	Study group (n=39)		Control group (n=41)		
	Median (range)	No. of patients with out-of-range tests (%)	Median (range)	No. of patients with out-of-range tests (%)	P value
aCL IgG (GPL)	3.13 (1.32–14.03)	3 (7.7)	2.42 (0.96–18.45)	1 (2.4)	0.003650
aCL IgM (MPL)	6.94 (1.90–26.40)	2 (5.1)	4.32 (1.9–28.73)	2 (4.9)	0.007892
aβ2GPI IgG (SGU)	4.10 (3.45–21.63)	1 (2.5)	0.76 (0.12–6.01)	0 (0)	0.000000
aβ2GPI IgM (SGU)	2.82 (1.44–11.70)	0 (0)	1.08 (0.44–3.64)	0 (0)	0.000000
LA	–	9 (27.7)	–	0 (0)	0.00547
ANA (titer)	–	1 (2.5)	NA	NA	NA
ANCA (titer)	–	23 (60.0)	NA	NA	NA

Values for aCL are expressed in units: GPL for IgG, MPL for IgM; values for aβ2GPI are expressed in SGU

aCL = anticardiolipin antibody, aβ2GPI = anti-β2-glycoprotein I, LA = lupus anticoagulant, ANA = antinuclear antibody, ANCA = antineutrophil cytoplasmic antibody

Table 4. aPL levels in women and men

	Women (n=6)	Men (n=33)	
	Median (range)	Median (range)	P value
aCL IgG (GPL)	2.70 (1.97–4.03)	3.20 (1.32–14.03)	0.192239
aCL IgM (MPL)	6.50 (3.26–15.86)	6.94 (1.90–26.40)	0.711541
aβ2GPI IgG (SGU)	3.84 (3.45–5.28)	4.10 (3.45–21.63)	0.310976
aβ2GPI IgM (SGU)	3.08 (1.70–3.64)	2.72 (1.44–11.70)	0.922474
LA	–	–	0.34520

Values for aCL are expressed in units: GPL for IgG, MPL for IgM; values for aβ2GPI are expressed in SGU

aPL = antiphospholipid antibodies, aCL = anticardiolipin antibody, aβ2GPI = anti-β2-glycoprotein I, LA = lupus anticoagulant

According to the ANA results, indirect fluorescence assay was positive in 13 patients; however, in only one patient did the ELISA test confirm positivity. In this patient SS-A/Ro-52 antibodies were significantly raised and SS-B and Scl-70 antibodies were slightly elevated. At the time of the study this patient was asymptomatic. In 23 patients ANCA assay was positive, but no further investigation was conducted.

DISCUSSION

Our data support the notion that antiphospholipid antibodies contribute to coronary artery disease and development of acute MI [2,7,12]. In a critical review of reports on the role of aPL in atherosclerosis, Artenjak et al. [14] presented data on the effect of aPL in three conditions: ischemic stroke, coronary artery disease, and peripheral artery disease. While patients with ischemic stroke had a negative correlation between aPL and atherosclerotic lesions, there is enough evidence to support a significant effect of aPL on the pathogenesis of peripheral artery disease. Nonetheless, the role of aPL in CAD remains unresolved. A positive correlation between aPL and CAD has been suggested [7,12] but not unanimously confirmed [8]. While aβ2GPI may play a role in the pathogenesis of atherosclerosis, aCL appear to have the highest correlation with progression of atherosclerosis. Other antibodies, e.g., anti-annexin, seem to be strongly associated with thrombosis [15] and progression of cardiovascular diseases [16].

The association between aPL, particularly aβ2GPI, and CAD may be explained by two types of mechanisms. Plaque formation caused by aβ2GPI is the effect of foam cell generation. aβ2GPI binding with oxLDL creates highly immunoreactive complexes, which subsequently promote accumulation of oxLDL in the macrophages [14] and lipid accumulation in the vessel wall. This mechanism can also be attributed to aCL [17]. We found no correlation between the number of atherosclerosis-affected vessels and the levels of aβ2GPI, aCL or LA. This may be explained by the volume or structure of the plaques. A positive correlation between aPL and atherosclerosis assessed by coronary artery calcium score was found in SLE patients [5].

Other mechanisms of aβ2GPI action are well known in antiphospholipid syndrome. Clot formation related to high levels of aβ2GPI is an effect of increased platelet adhesiveness, activation of the humoral pathway via the plasminogen system, inhibition of CRP, and the coagulation cascade. These mechanisms are associated not only with the progression of atherosclerotic plaque but also with higher MI incidence [7]. The coexistence of a genetic component is relevant to these mechanisms [18].

A fact that cannot be omitted when discussing aPL levels is that the immunoassays used in their detection may not be sufficiently accurate and reflect inter-method variation. Anticardiolipin antibodies may combine with aβ2GPI or RF antibodies/antigen and lead to false positive results [19]. Moreover, since the monoclonal antibodies in the assays are targeted at a single epitope, they do not fully reflect the diversity of subsets of polyclonal antibodies in the serum tested. In our study the threshold values for aPL assay were consistent with the current standard [20,21]. The concentration of aCL antibodies considered as medium and high titers of IgG and IgM correlated with clinical signs of antiphospholipid syndrome [21]. However, since there is no standard for distinguishing the threshold between moderate-high and low levels [22], defining the level that best corresponds to the risk of clinical manifestations is difficult [19]. Due to these drawbacks of current assays and standards, as well as to inter-laboratory variation, there is a clear need for new international units [23].

Lupus anticoagulant (LA) demonstrates a positive correlation with MI and ischemic stroke, especially in young patients without known autoimmunity [7]. Our data fully support that thesis, and the percentage of LA-positive patients in our study

is similar to those in previous studies. The role of LA in thromboembolic events is associated with its pro-thrombotic activity, demonstrating better correlation with these events than seen with other aPL [6]. Levine et al. [8] show that LA positivity may not result in increased risk of subsequent vascular events in patients without autoimmune disease; the negative correlation in their study might be the result of antiplatelet or anticoagulant treatment (their patients suffered from recurrent ischemic stroke). Despite that, in the same study, the patients with double positivity, i.e., positive to LA and aCL, had increased risk of vascular events within 2 years, even if they had received treatment.

Data on the role of antinuclear antibodies in atherogenesis are limited. It is possible that complexes built from anti-dsDNA antibodies, DNA and LDL may play a role in plaque formation. In an in vitro study, Kabakov et al. [24] showed that these complexes may augment lipid deposition in the vascular wall and may exert cytotoxic activity. SLE patients with excessive coronary calcifications, as detected by multi-detector computed tomography, have significantly higher ANA titers [5].

Antineutrophil cytoplasmic antibodies (ANCA) are usually associated with vasculitis, but their influence on CAD development is not known. In our study, CAD patients were characterized by increased ANCA levels, though this may be connected to vessel damage during revascularization procedures.

The final question is whether the presence of high aPL levels in CAD patients may influence the therapeutic strategy. In SLE patients with high aPL levels, prophylaxis with aspirin and anti-malarial drugs was suggested to be beneficial [25]. In CAD patients routinely treated with aspirin, chronic dual antiplatelet therapy may be advised, but further studies are required.

A major limitation of this study was that aPL assays were not repeated after 12 weeks. Also questionable is whether percutaneous procedure/revascularization therapy may cause an increase in the levels of autoantibodies. Thus, further studies are needed to clarify this issue.

CONCLUSIONS

In relatively young patients with coronary artery disease who underwent myocardial revascularization, the levels of antiphospholipid antibodies are significantly higher than in young healthy patients. Lupus anticoagulant is present in one-fourth of these patients. In this group, despite the classic well-known risk factors of coronary artery disease, autoimmunity may play an important role in atherosclerotic plaque formation and progression.

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