The prevalence and clinical significance of antiphospholipid antibodies in the patients with systemic sclerosis – preliminary report

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Abstract

Purpose: The aim of our study was to evaluate the prevalence of anticardiolipin and anti-β2-glikoprotein I (anti-β2GPI) antibodies in patients with systemic sclerosis (SSc) and to correlate the presence of these antibodies with clinical and serological features of the disease.

Material and methods: 22 patients (21 women and 1 man) fulfilling the ACR classification criteria of SSc were included into the study. In all SSc patients a detailed clinical evaluation including skin and internal organ involvement was performed. Moreover, the measurements of anti-topoisomerase I (anti-Scl-70) and anticentromere (ACA) antibodies were done in all patients studied. Anticardiolipin antibodies in IgM and IgG class and anti-β2GPI antibodies in IgM, IgG and IgA class were evaluated using ELISA kits (Hycor Biomedical and DiaSorin).

Results: Anticardiolipin antibodies were found in 10/22 (45.5%) patients with SSc, in 6/12 (50%) with diffuse SSc and in 4/10 (40%) with the limited SSc. Anticardiolipin antibodies in the IgG class were observed in 4/22 (18.2%) patients, and in the IgM class in 9/22 (40.9%) subjects. Anti-β2GPI antibodies were found in 9/22 patients (40.9%), of which 3/22 (13.6%) had antibodies in IgG class, 4/22 (18.2%) in IgM class and 3/22 (13.6%) in the IgA class. Anti-β2GPI antibodies were found exclusively in the patients in whom the anticardiolipin antibodies were also present. An association between the presence of antiphospholipid antibodies and internal organ involvement (pulmonary fibrosis, pulmonary hypertension and the alterations of oesophageal function) was not significant. No significant correlation was found between the presence of anticardiolipin or anti-β2GPI antibodies and the presence of anti-Scl-70 or ACA antibodies.

Conclusions: The results of our study indicate that the prevalence of anticardiolipin antibodies and anti-β2GPI antibodies is relatively high in patients with SSc. A more detailed assessment of the relationship between the presence of antiphospholipid antibodies and the clinical and serological features of SSc requires further studies on the larger group of patients and a several years of follow-up.

Key words: systemic sclerosis, antiphospholipid antibodies, anticardiolipin antibodies, anti-beta2-glycoprotein I antibodies.

Introduction

Antiphospholipid (aPL) antibodies have been investigated for many years. Their contribution to various clinical symptoms, mainly venous and arterial thrombosis, miscarriages and thrombocytopenia has been proved about 20 years ago. The occurrence of these clinical symptoms accompanied by the presence of anticardiolipin (aCL) antibodies and/or lupus anticoagulant (LAC) is called an antiphospholipid syndrome (APS). APS may be of the primary (there is no coincidence of another disease) or secondary origin (in the course of other diseases such as systemic connective tissue diseases, neoplasms, chronic infectious diseases).

Systemic sclerosis (SSc) belongs to the group of the connective tissue diseases. Vascular changes play the key role in the pathogenesis of SSc leading to the ischemia of different tissues and, as consequence, to a number of clinical complications [1-3]. Until recently the pathogenesis of these changes has remained unclear. There have been intensive studies on this subject, in the hope that, the knowledge of the origins of

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Table 1. **Clinical and laboratory characteristics of the patients with systemic sclerosis**

Patients with SSc (n= 22)	
Female	21
Male	1
Disease duration [years]	0.5-17 (mean 4.1)
Raynaud phenomenon no. (%)	21 (95.5)
Pulmonary fibrosis no. (%) a) early changes (HRCT) b) advanced fibrosis	15 (68.1) 5 (22.7) 10 (45.5)
Pulmonary hypertension no. (%)	8 (36.4)
Oesophageal dysmotility no. (%)	16 (72.7)
Renal involvement no. (%)	1 (4.5)
The presence of ACA no. (%)	9 (40.9)
The presence of anti-Scl-70 no. (%)	12 (54.5)

SSc=systemic sclerosis, HRCT=high resolution computed tomography, ACA=anticentromere antibodies, anti-Scl-70=anti-topoisomerase I antibodies

the disease may help to treat the patients with SSc more effectively [4].

APS is characterized by the presence of thrombotic changes in the venous and arterial vessels causing tissue ischemia. It has been shown that aCL affect the endothelial cells of the small vessels leading to the activation of the complement system with the following thrombosis. In view of the above theory special attention should be paid to the connection between the presence of aPL antibodies and SSc. The question if aPL antibodies may cause ischemic changes in the course of SSc [1,2], even when the criteria for the diagnosis of the secondary APS are not met, is of crucial importance. There have been only a few studies on the prevalence of the aPL antibodies in the patients with SSc. These studies have been based on the routine assessment of aCL antibodies in the IgG and IgM class while IgA class, which might have a great clinical significance, was not taken into account [5].

During the last few years new types of aPL antibodies, which do not fall into the range of a standard serological diagnostics, have been found. A lot of data indicate that the majority of the routine measurements allow to assess antibodies against the two serum phospholipid binding proteins: beta 2-glicoprotein I (β2GPI) and prothrombin [5,6,7]. Anti-β2GPI antibodies (anti-β2GPI) are mainly responsible for anticardiolipin activity in the serum of the patients with APS [8].

The aim of our study was to evaluate the prevalence of aCL antibodies and anti-β2GPI antibodies and to attempt to correlate the presence of these antibodies with the organ involvement in the patients with SSc.

Material and methods

The study included 22 patients (21 women and 1 man) with SSc (12 with diffuse SSc and 10 with its limited form) aged 23-71 years (mean 51.3 years). All patients met the criteria for the diagnosis of systemic sclerosis according to ARA (American College of Rheumatology, formerly American Rheumatism Association) [9]. In all studied patients a detailed clinical analysis has been performed with the assessment of skin score as well as joint and organ involvement. Moreover, the measurements of anti-topoisomerase I (anti-Scl-70) and anticentromere (ACA) antibodies using Pharmacia Diagnostics kits have been done. Anticardiolipin antibodies in IgM and IgG class and anti-β2GPI antibodies in IgM, IgG i IgA class were evaluated with ELISA kits (Hycor Biomedical and DiaSorin).

Clinical and laboratory data of the SSc patients are shown in *Tab 1*.

Statistical analysis

Statistical analysis was performed using the Mann-Whitney U test and the Fisher's exact test.

P values less than 0.05 were considered statistically significant.

Results

Anticardiolipin antibodies were found in 10 out of 22 (45.5%) patients with SSc, in 6 out of 12 (50%) with diffuse SSc and in 4 out of 10 (40%) with the limited SSc. Anticardiolipin antibodies in the IgG class were observed in 4 out of 22 (18.2%) patients, and in the IgM class in 9 out of 22 (40.9%) subjects. Anti-β2GPI antibodies were observed in 9 out of 22 patients (40.9%), of which 3/22 (13.6%) had antibodies in IgG class, 4/22 (18.2%) in IgM class and 3/22 (13.6%) in the IgA class. Anti-β2GPI antibodies were found exclusively in the patients in whom aCL antibodies were present. Consequently the presence of anti-β2GPI antibodies was confirmed in 9 out of 10 (90%) patients with aCL antibodies. Only in one woman with aCL antibodies there were no anti-β2GPI antibodies observed. The results of the measurements of aCL antibodies and anti-β2GPI antibodies are shown in *Tab. 2*.

In the patients with aCL antibodies and anti-β2GPI antibodies a statistical analysis with clinical symptoms and the presence of SSc-typical antibodies (anti-Scl-70 and ACA) using Fisher's test was done. Correlation between the presence of particular classes of antibodies with pulmonary fibrosis, pulmonary hypertension and the alterations of oesophageal function has also been performed. Our measurements showed no statistically significant correlations (for example the extent of the changes in the oesophagus did not correlate with anti-β2GPI IgG antibodies: $p=0.527$, whereas there was a trend of correlation with anti-β2GPI IgM antibodies: $p=0.087$). No statistical significance was found between the presence of aCL antibodies or anti-β2GPI antibodies and the presence of anti-Scl-70 or ACA.

Discussion

There have been reports that the presence of antiphospholipid (aPL) antibodies even in the asymptomatic patients (without thrombosis and miscarriages) indicates a greater risk of the development of vascular changes [10,11]. There are a few

Table 2. **The presence of aCL antibodies and anti-β2GPI antibodies in the patients with SSc**

Patient	aCL IgG	aCL IgM	a-β2GPI IgG	a-β2GPI IgM	a-β2GPI IgA
L.K.	9.8 (-)	9.7 (+)	2.9 (-)	28.66 (+)	1.6 (-)
J.S.	20.6 (++)	10.7 (+)	8.44 (-)	9.5 (-)	2.95 (-)
J.F.	38.6 (++)	5.8 (-)	42.21 (+)	5.95 (-)	2.5 (-)
S.P.	8.0 (-)	13.8 (+)	5.2 (-)	58.27 (+)	3.0 (±)
R.D.	7.3 (-)	12.4 (+)	7.8 (-)	8.0 (-)	15.34 (+)
A.J.	20.0 (++)	52.8 (+++)	24.44 (+)	20.18 (+)	2.5 (-)
A.Z.	4.3 (-)	9.9 (+)	40.01 (+)	10.65 (-)	2.8 (-)
H.P.	9.4 (-)	8.4 (+)	3.59 (-)	8.34 (-)	3.2 (±)
N.G.	21.6 (++)	5.4 (-)	6.74 (-)	10.8 (-)	8.5 (+)
Z.B.	7.4 (-)	50.3 (+++)	7.6 (-)	54.36 (+)	5.0 (+)

theories explaining the association between aPL antibodies and the development of vascular changes [12]. Antiphospholipid antibodies may bind to the phospholipids of the endothelial cells decreasing the release of prostacycline, which is the platelet aggregation inhibitor and a known vasodilating agent. It has also been proved that aPL antibodies inhibit protein C activation, causing an increased blood thrombosis. At present a lot of attention is paid to anti-β2GPI antibodies. It has been found that aPL antibodies do not react directly with phospholipids but instead act against β2GPI, a serum protein which has the capacity to bind to the anion phospholipids [13]. Ieko et al. [14] have found that β2GPI protects the tissue plasminogen activator (tPA) against its inhibitor (PAI-I), which decreases the fibrinolitic activity and leads to the increased thrombosis in the patients with anti-β2GPI antibodies. Anti-β2GPI antibodies in the IgG class may inhibit in vitro thrombin formation and are closely related to clinical manifestation of antiphospholipid syndrome [15]. Carreras et al. [16] have shown that anti-β2GPI antibodies have a much greater importance in the assessment of the risk of the development of thrombosis than aPL antibodies and may sometimes be detected in the absence of aPL antibodies in the standard tests.

Data concerning the frequency of the occurrence of aPL antibodies in the patients with SSc are scarce and contradicting. Different authors reported the presence of aCL antibodies in 0-63% of the patients with SSc [17,18]. Picillo et al. [4] have shown that there is an association between the presence of aCL antibodies and the worse course of SSc. However, Ihn et al. have observed a relation between the incidence of anti-β2GPI antibodies in the IgG class and pulmonary hypertension in the course of SSc [19]. In our study aCL antibodies were found in 10 (45.5%) patients with SSc, while anti-β2GPI antibodies were observed in 9 (40.9%) subjects. In 3 patients with anti-β2GPI antibodies in IgG class, there was no pulmonary hypertension contrary to the findings of Ihn et al. [19]. On the other hand similarly to Sherer et al. [20] there was no association between the presence of aCL antibodies and anti-β2GPI antibodies with systemic hypertension in our study. In the available papers there have been no data concerning the connection between aPL antibodies and the alterations in the lungs and digestive tract in the patients with SSc. In the majority of the patients (90%) a coincidence between aCL antibodies and anti-β2GPI antibodies was observed, which is confirmed by data from other studies.

The results of our study show that the prevalence of aCL antibodies and anti-β2GPI antibodies is not rare in the patients with SSc. A more detailed assessment of the relationship between the presence of aPL antibodies and organ involvement in the course of SSc requires further studies on the larger group of patients and a several years of follow-up.

Taking into account the data from other studies indicating the potential role of aPL antibodies in the development of vascular changes and the fact that vascular complications constitute a vital element of the pathogenesis of SSc, the results of our study suggest the use of anticoagulants as a standard element in the treatment of the patients with SSc. Recently there have been reports of a beneficial effect of statins in the patients with aPL antibodies, which may be explained in terms of the inhibitory effect of statins on endothelial cells simulated by anti-β2GPI antibodies [21].

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