CONCISE REPORTS

Anticardiolipin, anticentromere and anti-Scl-70 antibodies in patients with systemic sclerosis and severe digital ischaemia

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Abstract

Objective—Following observation of weakly positive anticardiolipin (aCL) antibodies in four of eight patients with systemic sclerosis (SSc) and severe digital ischaemia requiring amputation, the association between the presence of these and other antibodies and severe peripheral ischaemia in patients with SSc was examined.

Methods—ACL antibodies (IgG and IgM), anticentromere and anti-Scl-70 antibodies were measured in a further 60 patients with SSc over a one year period. Thirty one of the 68 patients in whom aCL antibodies were assayed had 'severe ischaemia', having suffered digital ischaemia severe enough to warrant amputation (13 patients), surgical debridement or admission for intravenous vasodilator therapy.

Results—There was no difference in aCL positivity between those with severe ischaemia and those without, nor between those who had amputations and those who had not. Three of the 31 patients (10%) with severe ischaemia had IgG and eight (26%) IgM aCL antibodies in weak to moderate titre compared to 10 (27%) and 6 (16%) respectively of the remaining patients ($p = 0.06$ for IgG and $p = 0.25$ for IgM, Fisher's exact test). Seventeen of the 31 patients (55%) with severe ischaemia were anticentromere antibody positive compared with nine of 37 (24%) without ischaemia ($p = 0.01$). Six patients with severe ischaemia had anti-Scl-70 antibodies compared with two of the 37 without ischaemia ($p = 0.08$).

Conclusions—The findings do not support an association between aCL antibodies and severe ischaemia in SSc, but confirm the previously reported association between anticentromere antibodies and severe peripheral ischaemia. Although anti-Scl-70 antibodies were present only in a small number of patients, there was also a tendency for these to be associated with severe ischaemia, suggesting that patients with either anticentromere or anti-Scl-70 antibodies should be considered at risk of digital loss.

A subgroup of patients with systemic sclerosis (SSc) develop severe peripheral ischaemia which can result in scarring, ulceration, and sometimes even gangrene. This peripheral ischaemia is due largely to the vascular changes typical of SSc (characteristically intimal thickening and fibrosis) occurring in the digital arteries. Following our observation of weakly positive anticardiolipin (aCL) antibodies in four of eight patients with SSc and severe digital ischaemia requiring amputation,¹ we investigated the possibility that the presence of these antibodies, albeit in low titre, might be associated with severity of digital ischaemia. This seemed plausible because of the well-recognised associations between aCL antibodies and vascular disease.² We also measured anticentromere and anti-Scl-70 (antitopoisomerase I) antibodies, both highly specific for SSc, to see whether we would confirm the previously reported association between anticentromere antibody and digital loss,³ or demonstrate a relationship between the presence of anti-Scl-70 antibodies and severe ischaemia.

Patients and methods

ACL antibodies were assayed in 60 unselected patients in whom a clinical diagnosis of SSc had been made and who attended the Rheumatic Diseases Centre over a one year period. This meant that when the eight previously investigated patients were included, a total of 68 patients (10 men, 58 women, median age 49 years, range 19–79 years) were studied. The median duration of Raynaud's in these 68 patients was 12 years, range one month to 49 years. Sixty two fulfilled the ARA criteria for SSc.⁴ Of the six others, all suffered from Raynaud's and four were anticentromere antibody positive with at least one of the following clinical features: sclerodactyly, digital pitting, calcinosis or nail-fold capillary changes. Both the remaining patients had sclerodactyly, while one had nail-fold capillary changes and oesophageal dysmotility, and the other had extensive gastro-intestinal involvement of her disease.

Case-sheets of all patients were reviewed to determine whether or not they could be classified as having had 'severe ischaemia', defined as being severe enough to warrant amputation, surgical debridement or admission for intravenous vasodilator therapy.

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Antibodies to cardiolipin were analysed using an ELISA technique as previously described.⁵ All results were calculated using the same standard sera. The values of the individual isotypes were expressed as IgG and IgM aCL: the upper limits of the normal reference ranges were five and three units respectively.

Anticentromere antibodies were identified by their characteristic staining pattern on indirect immunofluorescence using a HEp-2 cell line as the substrate.⁶ Anti-Scl-70 antibodies were identified by Ouchterlony double diffusion using rabbit thymus extract as the antigen source. The identity of the precipitation line was established using a characterised reference serum standardised against a monospecific positive serum control obtained from the ANA Reference Laboratory, Centres for Disease Control, Atlanta, USA.

As smoking, treatment with prednisolone, and presence of other antibodies (anti-Ro, anti-La, anti-RNP, and anti-Sm) might act as confounding factors by being associated with ischaemia, these too were documented for each patient.

The Mann-Whitney U test was used to compare durations between groups. Odds ratios with exact confidence intervals were used to examine individual risk factors for ischaemia and amputation. Fisher's exact test was used to test significance. Multiple logistic regression was then used to adjust for the effects of smoking and duration.

Results

Thirty one of the 68 patients had 'severe ischaemia', and thirteen of these had required amputations.

Table 1 ACL, anticentromere and anti-Scl-70 antibodies, smoking habit and steroid therapy in patients with and without severe peripheral ischaemia

	Severe peripheral ischaemia (%) n = 31	No severe peripheral ischaemia (%) n = 37	Odds ratios (95% confidence intervals)	Adjusted odds odds π (95% confidence intervals)
aCL				
IgG+	3 (10)	10 (27)	0.3 (0.05,1.3)	0.3 (0.1,1.4)
IgM++	8 (26)	6 (16)	1.8 (0.5,7.2)	1.5 (0.4,5.4)
Anticentromere	17 (55)	9 (24)	3.8 (1.2,12.1)*	3.9 (1.3,11.8)**
Anti-Scl-70	6 (19)	2 (5)	4.2 (0.7,45.0)	8.0 (1.3,48.1)**
Prednisolone	6 (19)	9 (24)	0.7 (0.2,2.8)	1.0 (0.3,3.6)
Smoking	15 (48)	7 (19)	4.0 (1.2,14.0)*	

* p = 0.01 between groups.

** p = 0.02 between groups.

+ in low to moderate titre (>5 <50 units).

++ in low to moderate titre (>3 <30 units).

π for duration of disease and smoking.

Table 2 ACL, anticentromere and anti-Scl-70 antibodies, smoking habit and steroid therapy in patients with and without amputations

	Amputations (%) n = 13	No amputations (%) n = 55	Odds ratios (95% confidence intervals)	Adjusted odds ratios π (95% confidence intervals)
aCL				
IgG+	1 (8)	12 (22)	0.3 (0.006,2.5)	0.4 (0.04,3.5)
IgM++	4 (31)	10 (18)	2.0 (0.4,9.1)	1.7 (0.4,7.9)
Anticentromere	10 (77)	16 (29)	8.1 (1.7,50.2)*	7.6 (1.7,33.8)**
Anti-Scl-70	1 (8)	7 (13)	0.6 (0.01,5.2)	1.0 (0.1,9.6)
Prednisolone	3 (23)	12 (22)	1.1 (0.2,5.1)	1.6 (0.3,8.0)
Smoking	7 (54)	15 (27)	3.1 (0.7,13.0)	

* p = 0.002 between groups.

** p = 0.008 between groups.

+ in low to moderate titre (>5 <50 units).

++ in low to moderate titre (>3 <30 units).

π for duration of disease and smoking.

Immunological results, including odds ratios with confidence intervals, are summarised in tables 1 and 2. Three of the 31 patients (10%) with 'severe ischaemia' had IgG aCL and eight (26%) IgM aCL antibodies in weak to moderate titre (>5 and <50 units for IgG; >3 and <30 units for IgM) compared with 10 (27%) and six (16%) respectively of the 37 remaining patients (p = 0.06 for IgG and p = 0.25 for IgM). Nor was there any difference in aCL positivity between those who had amputations and those who had not (p = 0.23 for IgG and p = 0.26 for IgM).

Seventeen of the 31 patients (55%) with severe ischaemia were anticentromere antibody positive compared with nine of 37 (24%) without ischaemia (p = 0.01). Ten of the 13 (77%) with amputations had these antibodies compared with 16 of 55 (29%) without (p = 0.002). Six patients with severe ischaemia had Scl-70 antibodies compared with two of the 37 without ischaemia (p = 0.08).

Regarding the presence of antibodies to Ro, La, Sm and RNP, one of the patients had both anti-Ro and anti-La antibodies, while one had anti-La and two had anti-Ro. Four patients had RNP antibodies. Of these eight patients only one (with RNP antibodies) had severe ischaemia.

Patients with severe ischaemia had a longer disease duration (median 13 years, range 2.5 to 49 years) than those without (median 11 years, range one month to 32 years, p = 0.09). Similarly, patients who had amputations had a longer disease duration (median 16 years, range four years to 49 years) than those without (median 10 years, range one month to 32 years, p = 0.03).

Fifteen of the 31 patients (48%) with severe ischaemia were smokers compared with seven of the 37 (19%) without (p = 0.01). Seven of the 13 patients (54%) with amputations were smokers compared with 15 of 55 (27%) without (p = 0.07).

Prednisolone therapy was not associated with an increased risk of severe ischaemia or amputation. Fifteen patients were on prednisolone, six of whom had severe ischaemia and three had amputations.

After adjusting for disease duration and smoking habit, anticentromere antibody remained significantly associated with severe ischaemia and amputation, and the association between anti-Scl-70 and severe ischaemia became statistically significant.

Discussion

ACL antibodies have been reported in a wide variety of infective and inflammatory states.⁷ While SSc is not generally regarded as an inflammatory disease, we have found a low-grade vasculitis in digits amputated from patients with SSc and severe digital ischaemia.¹ However, it is not known whether inflammation can lead to the production of aCL antibodies, or whether these antibodies can themselves, in the longer term, contribute to the pathogenesis of inflammatory vascular disease. Our results do not support an

important pathogenic role for aCL antibodies in the severe digital ischaemia associated with SSc. Although 23 of our 68 patients (34%) were found to have aCL antibodies in weak to moderate titre, patients with severe ischaemia were no more likely than those without to have these antibodies. No patient had the high titres typically associated with the vasculopathy of the antiphospholipid syndrome. In our laboratory such patients would typically have aCL IgG levels in excess of 50 units/ml.

Several other authors have investigated the prevalence of aCL antibodies in SSc with varying results.⁸⁻¹² In 1986 Seibold *et al* reported aCL antibodies, predominantly in low titre, in 11 of 35 patients with SSc.⁸ Subsequently these antibodies were detected in seven of 28 patients, and in this study an association with significant visceral disease was suggested.⁹ Particularly relevant to our own findings is that Passaleva *et al* reported aCL antibodies in 41% of 17 patients with limited cutaneous SSc and finger ulceration, but apparently the presence of these antibodies did not correlate with the presence of vascular lesions.¹⁰ Thirteen of 40 patients with SSc were found to have aCL antibodies by Katayama *et al*,¹¹ but another Japanese study reported that none of 60 SSc patients were aCL antibody positive.¹² The varying results of these studies will in part relate to the well recognised interlaboratory variability in aCL methodology. Our study is the first to examine whether a relationship exists between the presence of these antibodies and whether or not a patient has severe digital ischaemia as defined above.

Our results emphasise the recognised relationship between anticentromere antibodies and the risk of digital ischaemia.³ Patients with severe ischaemia, and especially those having had amputations, had a higher prevalence of these antibodies than the patients with milder ischaemia.

Although anti-Scl-70 antibodies were only present in a small number of our patients, there was a tendency for these also to be associated with severe peripheral ischaemia (although not with the severest ischaemia as defined by the necessity for amputation). This finding was of interest because antibodies to Scl-70 tend to be associated with diffuse, rather than limited cutaneous, SSc¹³ and patients with diffuse disease are often considered to have less severe digital ischaemia than those with the limited cutaneous (CREST) variant. However, Steen *et al*¹³ reported that in patients with diffuse SSc, anti-Scl-70 was possibly associated with peripheral vascular disease as the subset who were anti-Scl-70 positive were more likely to have digital tip ulcers and pitting scars than

those who were anti-Scl-70 negative. In her study no correlation between anti-Scl-70 and severe ischaemia was looked for.

In contrast to Wigley *et al*,³ we found that smoking and longer duration of Raynaud's were associated with severe ischaemia and amputation, but adjustment for these did not significantly affect our conclusion that anticentromere and anti-Scl-70 antibodies are risk factors for this ischaemia.

The high frequency of amputation in our patient group (13 of 68 patients or 19%) reflects our department's interest in vascular disease and is similar to that of 20-4% reported by Wigley *et al*.³

Whatever the pathogenic role of aCL antibodies may be, our findings suggest that these are unlikely to be important in the digital ischaemia associated with SSc. Antibodies to Scl-70 as well as anticentromere antibodies may be associated with severe digital ischaemia. Patients with either of these antibodies should therefore be considered a group at high risk of digital loss and efforts to prevent and treat digital ischaemia should be particularly rigorous in these patients.

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