Detection of IgE-autoantibodies to nuclear antigens in patients with systemic sclerosis and analysis of their clinical relevance

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Abstract

Objective

Antinuclear antibodies (ANA) of the IgE-type have been described in several connective tissue disorders (CTD) but not yet in systemic sclerosis (SSc). Aim of the study was, therefore, to establish an ELISA for the demonstration of IgE-auto-antibodies to topoisomerase-I (topo-I) and the centromeric proteins A and B (CENP-A/B), to assess their prevalence and reactivity in SSc and to analyse their clinical relevance.

Methods

One hundred fifty-one patients with SSc and 88 with CREST syndrome, 291 patients with other CTD, and 23 patients with fibromyalgia syndrome (FM) as a control collective were included into the study. Patients' sera were analysed by an in-house-ELISA for IgE autoantibodies against topo-I and CENP-A/B using recombinant antigens. Patients were assessed for median Rodnan skin score(mRSS), different organ and cutaneous manifestations.

Results

Of the patients with CREST syndrome, 67% had IgE-anti-CENP-A- and 77% IgE-anti-CENP-B-antibodies. IgE-anti-topo-I antibodies were found in 56% of patients with SSc. Prevalence and reactivity were significantly higher in CREST and SSc, respectively, than in other CTD or FM. IgE-reactivity strongly correlated with IgG-antibody reactivity. In CREST syndrome, IgE-anti-CENP-A (but not CENP-B)-antibodies were significantly higher and more prevalent in patients with skin ulcers, high mRSS, and more than four organ manifestations. They did not correlate with blood eosinophil counts. In contrast, for IgE-anti-topo-I antibodies no correlation with clinical manifestations was observed.

Conclusion

IgE-autoantibodies against CENP-A/B and topo-I occur in SSc underlining the concept that SSc may be a T helper cell type 2 mediated disease. IgE-anti-CENP-A-antibodies correlated with disease activity, but this has to be confirmed in larger studies.

Key words

systemic sclerosis, IgE, autoantibodies, DNA topoisomerase-I, CENP-A protein, CENP-B protein

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Received on July 6, 2023; accepted in revised form on November 28, 2023.

Competing interests: none declared.

Introduction

Systemic sclerosis (SSc) is an immune-mediated rheumatic disease, characterised by fibrosis of the skin and visceral organs (1, 2). Based on clinical and serological criteria SSc can be classified as diffuse cutaneous systemic sclerosis (dsSSc) and limited cutaneous systemic sclerosis (lcSSc) according to the extent of skin involvement (1, 3). CREST syndrome is a specific clinical manifestation of lcSSc comprising calcinosis, Raynaud's phenomenon, oesophageal motility disorder, sclerodactyly and telangiectasia (4). The most relevant autoantibodies measured in SSc are anti-Scl70-antibodies directed against topoisomerase-I (topo-I) in SSc and anticentromere antibodies (ACA) in CREST syndrome (5, 6). In all connective tissue diseases (CTD) the diagnostically relevant antinuclear antibodies (ANA) are of the IgG-type. However, in patients with systemic lupus erythematosus (SLE) also IgE autoantibodies have been described about five decades ago by Miyawaki *et al*. (7). Thereupon, Permin *et al*. demonstrated the presence of IgE-autoantibodies also in rheumatoid arthritis and discussed the pathogenic role of these antibodies in autoimmunity (8). Since then, ANA of the IgE-type reacting with different antigens such as nucleosomes, dsDNA, Sm, RNP, SSA/Ro, SSB/La in SLE-patients have been described in several studies (9, 10, 11). Autoreactive IgE-antibodies in other CTD have been hardly reported (8, 12, 13), in SSc they have not yet been analysed. The aim of the present study was, therefore, to investigate the occurrence of IgE-autoantibodies against the most relevant antigens in patients with SSc, namely towards topo-I and the centromere proteins (CENP) A and B. Furthermore, the prevalence of these autoantibodies in other connective tissue diseases and control subjects was assessed as well as their relevance with respect to different clinical manifestations and parameters.

Patients

One hundred and fifty-one patients with SSc (97 female, 54 male) and 88 patients with CREST syndrome (88 female, 5 male) were included in the study.

In all patients, diagnosis was in accordance with the 2013 Classification Criteria for SSc (14). All patients were assessed for the modified Rodnan skin score (mRSS) and different organ manifestations. Data collected included nailfold videocapillaroscopy (NVC), kcv left ventricular ejection fraction (LVEF in %) and systolic pulmonary arterial pressure (in mmHg) on echocardiography, resting and 24 h Holter-electrocardiograms, abnormal cardiac magnetic resonance imaging (MRI) or pulmonary arterial hypertension on right heart catheterisation (RHC), forced vital capacity (FVC) and diffusion capacity of the lungs for carbon monoxide (DLCO, % of predicted values), as well as high resolution computed tomography of the lungs. Moreover, N-terminal pro brain natriuretic peptide (NT-proBNP), and troponin-I were determined for monitoring cardiac damage as recently described (15, 16). Esophagogastroduodenoscopy was performed for the verification of gastrointestinal manifestations. Myopathy was defined by evidence of proximal muscle weakness in the presence/absence of muscle enzyme elevations with at least one abnormal objective testing such myopathic findings on electromyography (EMG) or inflammation, fibrosis or necrosis on muscle biopsy. Differential blood count and liver enzymes were determined for the evaluation of haematological or hepatological diseases, C-reactive protein (CRP) and C3/C4 complement were measured for the evaluation of inflammatory activity. The presence of nephrotic syndrome, persistent proteinuria more than 500–1000 mg/day with or without haematuria, unexplained acute kidney injury, or chronic kidney disease was the basis for the diagnosis of renal involvement.

Of the 151 SSc patients, 95 suffered from diffuse cutaneous and 56 from limited cutaneous sclerosis. All 151 patients were tested for anti-topo-I antibodies of the IgG-type, IgE-antibodies were analysed in 116 patients.

All patients with CREST syndrome suffered from limited cutaneous systemic sclerosis. Sera from all 88 patients were analysed for anti-CENP-A/B antibodies of the IgG-type, 73 of

Table I. a: Frequency of IgE- and IgG-anti-CENP-A/B antibodies in patients with different connective tissue diseases and controls.

	IgE-antibodies	IgG-antibodies
	number positive / number tested (%)	
Anti-CENP-A antibodies		
CREST syndrome	49 / 73 (67)	68 / 88 (77)
SSc (dcSSc and lcSSc)	2 / 20 (10) ****	13 / 151 (9) ****
MCTD	0 / 24 (0) ****	7 / 41 (17) ****
Sjögren's syndrome	5 / 28 (18) ****	25 / 118 (21) ****
SLE	5 / 21 (24) ****	19 / 110 (17) ****
FM	0 / 23 (0) ****	1 / 23 (4) ****
Anti-CENP-B antibodies		
CREST syndrome	57 / 73 (78)	69 / 88 (78)
SSc (dcSSc and lcSSc)	5 / 20 (25) ****	15 / 151 (10) ****
MCTD	0 / 24 (0) ****	3 / 41 (7) ****
Sjögren's syndrome	6 / 28 (21) ****	19 / 118 (16) ****
SLE	6 / 21 (29) ****	12 / 110 (11) ****
FM	1 / 23 (4) ****	0 / 23 (0) ****

*** $p<0.001$ **** $p<0.0001$ as compared to patients with CREST syndrome.

b: Prevalence of IgE-anti-CENP antibodies in 73 patients with CREST syndrome in relation to patients' therapy.

Kind of therapy	Number of patients tested	Anti-CENP IgE-antibodies	
		Anti-CENP-A	Anti-CENP-B
		Number (%) positive	
Without any therapy	57	40 (70)	50 (88)
Only with steroids	5	2 (20)*)	1 (20)***)
With immunosuppressive agents [a]	11	7 (64)*)	5 (55)**)

Significant as compared to patients without therapy: * not significant; ** $p<0.05$, *** $p<0.01$.
[a] azathioprine, methotrexate, cyclophosphamide, mycophenolate.

them were tested additionally for IgE-antibodies.

Clinical data of the patients including therapy and time between first diagnosis and time of serological diagnosis are given in Supplementary Table S1.

Furthermore, sera from 132 patients with systemic lupus erythematosus (SLE; 117 female, 15 male; age: median 35, range 13–74 years), 118 patients with Sjögren's syndrome (109 female, 9 male; age: median 51, range 13–81 years), 41 patients with mixed connective tissue disease (MCTD; 36 female, 5 male; age: median 36, range 10–72 years) and 23 patients with fibromyalgia (FM; 16 female, 7 male; age: median 50, range 25–62 years) as autoantibody negative control cohort were investigated. All patients were seen by one of the authors (ACP, JH) in the Rheumatological Outpatient Clinic of the Department of Internal Medicine, University Hospital in Tübingen, and in all of them diagnoses were based on current international classification criteria. The study was approved by the local ethics committee (No. 076/212BO1; 647/2016BO2) and was performed according to the Helsinki guidelines, all patients provided written informed consent.

Material and methods

Antigens and antibodies used in enzyme-linked immunosorbent assay (ELISA)

All recombinant antigens were purchased from Diarect AG, Freiburg (Germany). The recombinant antigens utilised were DNA topoisomerase-I (Scl-70; full length), Centromere Protein A (CENP-A) and Centromere Protein B (CENP-B). As secondary antibodies, peroxidase-conjugated AffiniPure Goat Anti-Human IgG (Jackson ImmunoResearch, West Grove, USA), and Goat Anti-Human IgE (ε-chain specific)- antibodies (Sigma-Aldrich, München, Germany) were used.

ELISA

IgG antibodies to topoisomerase-I were detected by an in-house ELISA as described (17, 18, 19); the demonstration of anti-CENP antibodies was adapted accordingly. For the assessment of IgE-antibodies optimal antigen and serum dilutions were determined by serial dilutions prior to the study (data not shown). 96-well microtiter plates (Nunc™ Maxisorp, Thermo Fisher Scientific, Roskilde, Denmark) were coated with the antigens topo-I, CENP-A and CENP-B in a concentration of 0.5µg/ml for the detection of IgE- and of 0.33µg/ml for the detection of IgG-antibodies. Patient's sera were diluted 1:2 for IgE- and 1:1,000 for IgG-antibody testing. Bound antibodies were detected using peroxidase-conjugated anti-human IgE-antibodies diluted 1:500 or peroxidase-conjugated anti-human IgG-antibodies diluted 1:3,000. As controls, one serum with a high and one serum with a moderate autoantibody reactivity and an antibody negative serum were applied to each test. All tests were performed in duplicates. Results are given as arbitrary units (AU) defined as optical density multiplied by 1,000. The normal ranges for the different autoantibodies were determined using sera from the antibody negative FM patients. The upper limit of normal range was defined by adding the average of the AU to the threefold standard deviation.

With this test system, an intra-assay coefficient of variation of 5.5% and an inter-assay coefficient of variation of 9.3% were achieved.

Statistics

GraphPad Prism version 9.2.0 was used for statistical analyses. Non-parametric tests were applied. Mann-Whitney-U-Test was performed for the analysis of unpaired reactivity values. Correlation of non-parametric data of IgG- and IgE-antibodies was performed using the Spearman correlation coefficient. Chi-Squared-Tests were used in the analysis of paired, nominal attributes of organ manifestations and antibodies without normal distribution. Mann-Whitney U-tests were performed for the analysis of paired reactivity val-

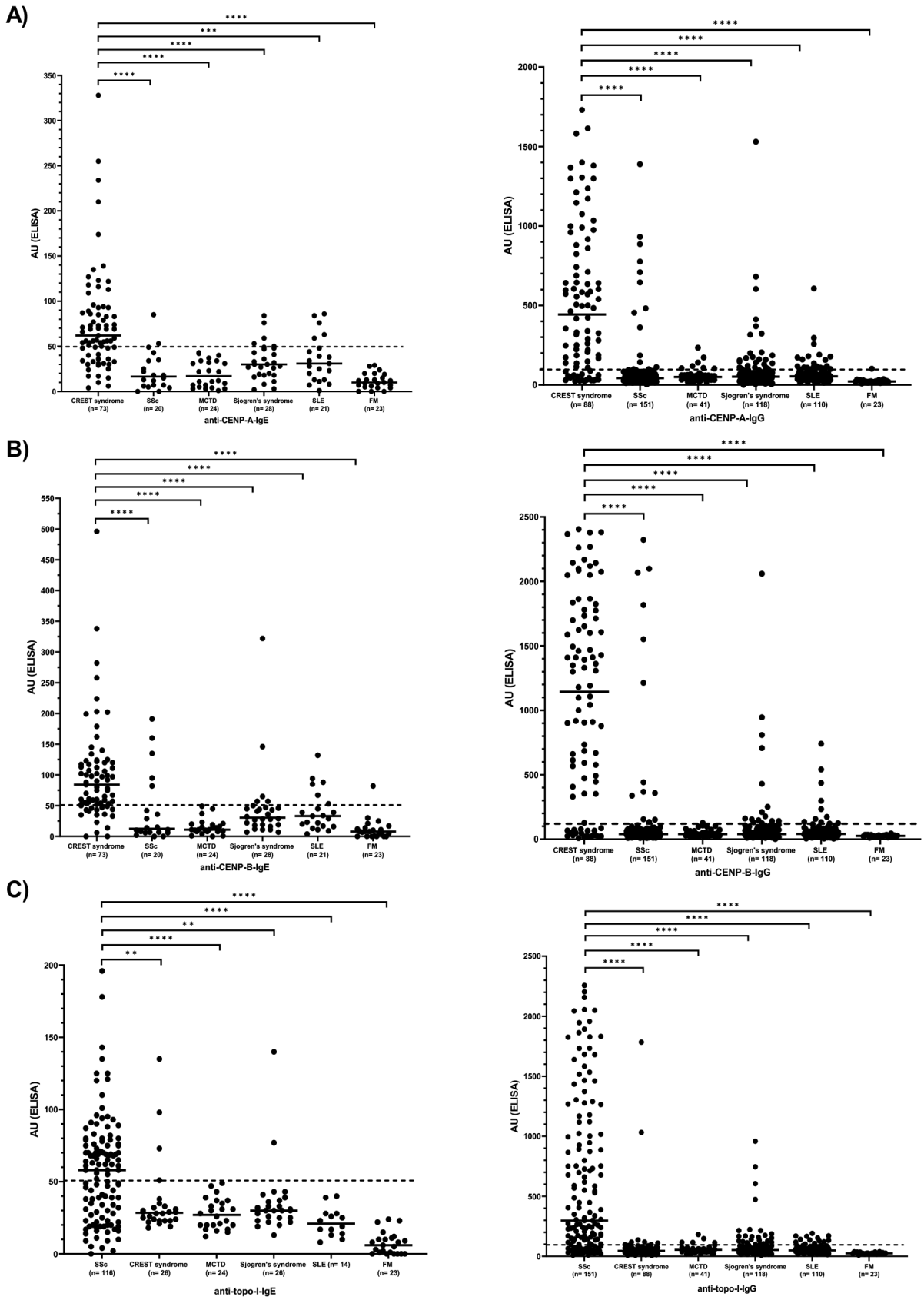

Fig. 1. IgE- and IgG-antibody-reactivity to CENP-A and -B (**A, B**), and topo-I (**C**) in patients with different CTD.
SSc: systemic sclerosis (dcSSc and lcSSc); MCTD: mixed connective tissue disease; SLE: systemic lupus erythematosus; FM: fibromyalgia syndrome. Individual values are given. ----: threshold value.
* $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$.

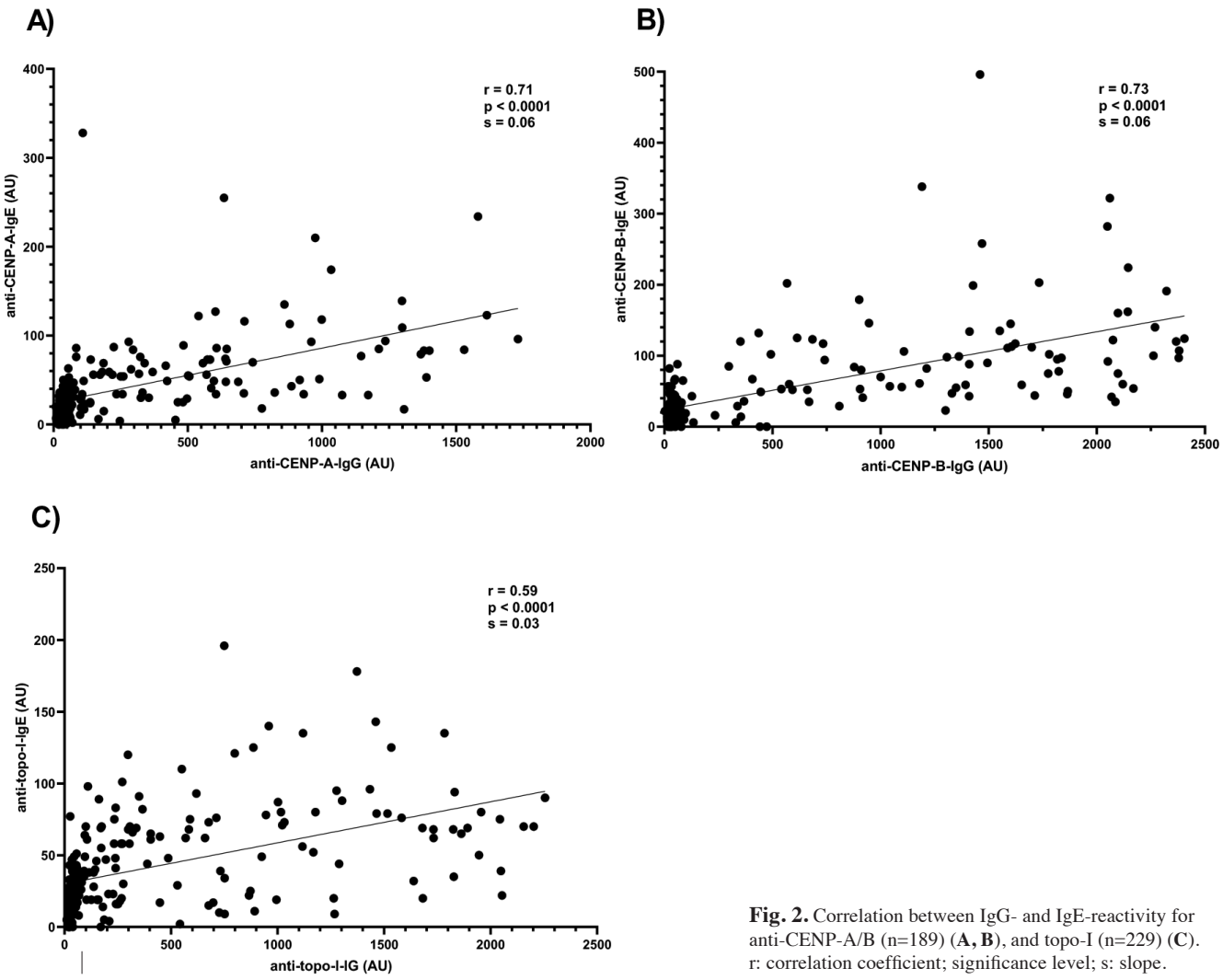

Fig. 2. Correlation between IgG- and IgE-reactivity for anti-CENP-A/B (n=189) (**A, B**), and topo-I (n=229) (**C**). r: correlation coefficient; significance level; s: slope.

ues grouped by the attributes of organ manifestations. *p*-values <0.05 were considered statistically significant.

Results

Demonstration of IgE-antibodies to CENP and topoisomerase-I in patients with SSc and other connective tissue disorders

It became clearly evident that antibodies to CENP-A/B and topo-I can belong to the IgE-class and that these antibodies are strongly associated with CREST syndrome and SSc, respectively. Thus, 67% of the patients with CREST syndrome had anti-CENP-A- and 78% anti-CENP-B antibodies of the IgE type (Table Ia) showing a significantly higher prevalence than in patients with SSc or other CTD. Also, reactivity of anti-CENP-A- and -B-antibodies of the IgE- (and IgG-type) was significantly higher in patients with CREST syndrome than in patients with other CTD (Fig. 1A-B). There was a strong correlation between the anti-CENP-A- and -B-reactivity of the IgG- and IgE-type (anti-CENP-A: spearman's correlation coefficient r=0.708 [confidence interval 0.626–0.774], $p<0.0001$; anti-CENP-B: spearman's correlation coefficient r=0.729 [confidence interval 0.652–0.791], $p<0.0001$) (Fig. 2A-B). IgE-antibodies were in most instances associated with IgG-antibodies (of the 49 patients with anti-CENP-A IgE-antibodies 48 [98%] and of the 57 patients with anti-CENP-B IgE-antibodies 56 [98%] had also IgG-antibodies) (Suppl. Table S2).

Prevalence and reactivity of the IgE-anti-CENP-antibodies was also analysed in relation to the kind of therapy. Prevalence of anti-CENP-B antibodies of the IgE-type was significantly lower in patients under steroids or immunosuppressive therapy than in patients without therapy (Table Ib), and also reactivity was in these patients significantly lower (median without therapy: 61 AU, with steroids: 23 AU [$p<0.01$], with immunosuppressive therapy: 35 AU [$p<0.05$]; not shown). In contrast, anti-CENP-A antibodies of the IgE type did not differ significantly between patients with or without therapy neither with respect to prevalence (Table Ib) nor reactivity (not shown), which may, however, be due to the low number of patients.

Reactivity and prevalence of anti-topo-I antibodies of the IgE type were significantly higher in patients with SSc compared to patients with CREST syndrome or other CTD (Fig. 1C, Table IIa). Thus, 56% of the SSc patients had IgE-anti-topo-I antibodies as compared

to 15% of patients with CREST and 8% of patients with Sjögren's syndrome. In most instances, anti-topo-I antibodies of the IgE-type were associated with IgG-antibodies (Suppl. Table S3), *i.e.* of the 65 patients with IgE-antibodies 62 (95%) had also IgG-antibodies. Only three patients had isolated IgE anti-topo-I antibodies. Accordingly, there was a significant correlation between anti-topo-I reactivity of the IgG- and IgE-type in the SSc patients (spearman's correlation coefficient r=0,589 [95% confidence interval (CI) 0.494–0.670], p<0,0001) (Fig. 2C).

Prevalence of IgE-anti-topo-I antibodies was similar in SSc patients without therapy or under prednisolone or immunosuppressive agents but significantly lower in patients after autologous stem cell transplantation (aSCT) (Table IIb). Also, reactivity was significantly lower in the patients after stem cell transplantation than in patients without therapy (median AU 25 *vs.* 43.5; p<0.05).

Table II. a: Frequency of IgG- and IgE-anti-topo-I antibodies in patients with different connective tissue diseases and controls.

	Anti-topo-I antibodies	
	IgE-type	IgG-type
	Number positive / number tested (%)	
SSc (dcSSc and lcSSc)	65/116 (56)	108/151 (72)
CREST syndrome	4/26 (15)****	3/88 (3)****
MCTD	0/24 (0)****	2/41 (5)****
Sjogren's syndrome	2/26 (8)****	19/118 (16)****
SLE	0/14 (0)****	10/110 (9)****
FM	0/23 (0)****	0/23 (0)****

**** p<0.0001 as compared to patients with SSc.

b: Prevalence of IgE-anti-topo-I antibodies in 116 patients with SSc in relation to patients' therapy.

Kind of therapy	Number of patients tested	Number (%) IgE-anti-topo-I positive
Without any therapy	32	20 (63)
Only with steroids	10	8 (80)*
With immunosuppressive agents [a]	62	36 (58)*
After autologous stem cell transplantation	12	1 (8)**

Significant as compared to patients without therapy: * not significant; ** p<0.01.
[a] azathioprine, methotrexate, cyclophosphamide, mycophenolate.

Correlation of IgE-autoantibodies in SSc with clinical manifestations and disease activity

IgE-anti-CENP-A/B reactivity and prevalence was correlated with clinical manifestations including mRSS, number of organ manifestations, cutaneous, pulmonary, gastrointestinal and cardiac manifestation in 50 patients with CREST syndrome (Fig. 3, Suppl. Table S4). Reactivity of IgE-anti-CENP-A antibodies significantly increased with the number of organ manifestations as well as the mRSS score and also for the anti-CENP-B antibodies this trend (p=0.07) was observed (Fig. 3, Suppl. Table S4). Moreover, there was a significant increase in reactivity and prevalence of IgE-anti-CENP-A antibodies in patients with as compared to patients without cutaneous ulcerations. This difference was not as evident for anti-CENP-B antibodies. For other organ manifestations including lung fibrosis and vasculopathy no significant differences were observed (Suppl. Table S4), but this may be due to the low number of patients in distinct groups.

Since it had been reported that the presence of skin ulcers in SSc is correlated with blood eosinophilia (20) we also analysed IgE-anti-CENP-A/B reactivity in relation to eosinophil counts (Suppl. Table S2). However, there was no significant difference between patients with eosinophils >300/mm^3 as compared to patients with eosinophil counts <300/mm^3 (p=0.45 for IgE-anti-CENP-A and p=0.78 for IgE-anti-CENP-B), and there was also no significant correlation between absolute eosinophil counts and IgE-anti-CENP-A/B reactivity (r=0.09 and r=-0.07, respectively). Moreover, in this series patients with skin ulcers had no higher eosinophil counts (median 189/mm^3) as compared to patients without ulcers (median 120/mm^3; p=0.38), and also prevalence of eosinophil counts >300/mm^3 was not significantly higher in the group of patients with ulcers (25%) as compared to that without ulcers (14%; p=0.38).

Also, reactivity and prevalence of anti-topo-I antibodies of the IgE-type was correlated with different clinical manifestations in SSc patients (n=63). However, there was no significant difference comparing patients with dcSSc or lcSSc as well as those without or with distinct organ manifestation including lung fibrosis or vasculopathy (Fig. 4, Suppl. Table S5).

Correlation of IgE autoantibodies in SSc with the time between first diagnosis and serological analysis

The prevalence of autoantibodies to topo-I and CENP-A/B did not correlate with the time between first diagnosis and time of serological diagnosis (Suppl. Table S6), and there was also no correlation between the IgE-antibody reactivity and time after first serological diagnosis (anti-CENP-A-IgE: r=-0.045, p=0.76; anti-CENP-B-IgE: r=0.10, p=0.47; anti-topo-I-IgE: r=-0.002, p=0.99).

Follow up of anti-topo-I IgE-reactivity in the course of disease in six SSc patients

Analysing the course of anti-topo-I IgE antibodies in six SSc patients with different therapeutic regimes for up to 180 months, no clear pattern was observed. There was some evidence that patients after aSCT had lower antibody reactivity, but this seemed to increase again at later time points (Suppl. Fig. S1).

Discussion

To our knowledge this is the first study that demonstrates the presence of IgE-antibodies to CENP-A and -B as well as topoisomerase-I in patients with SSc

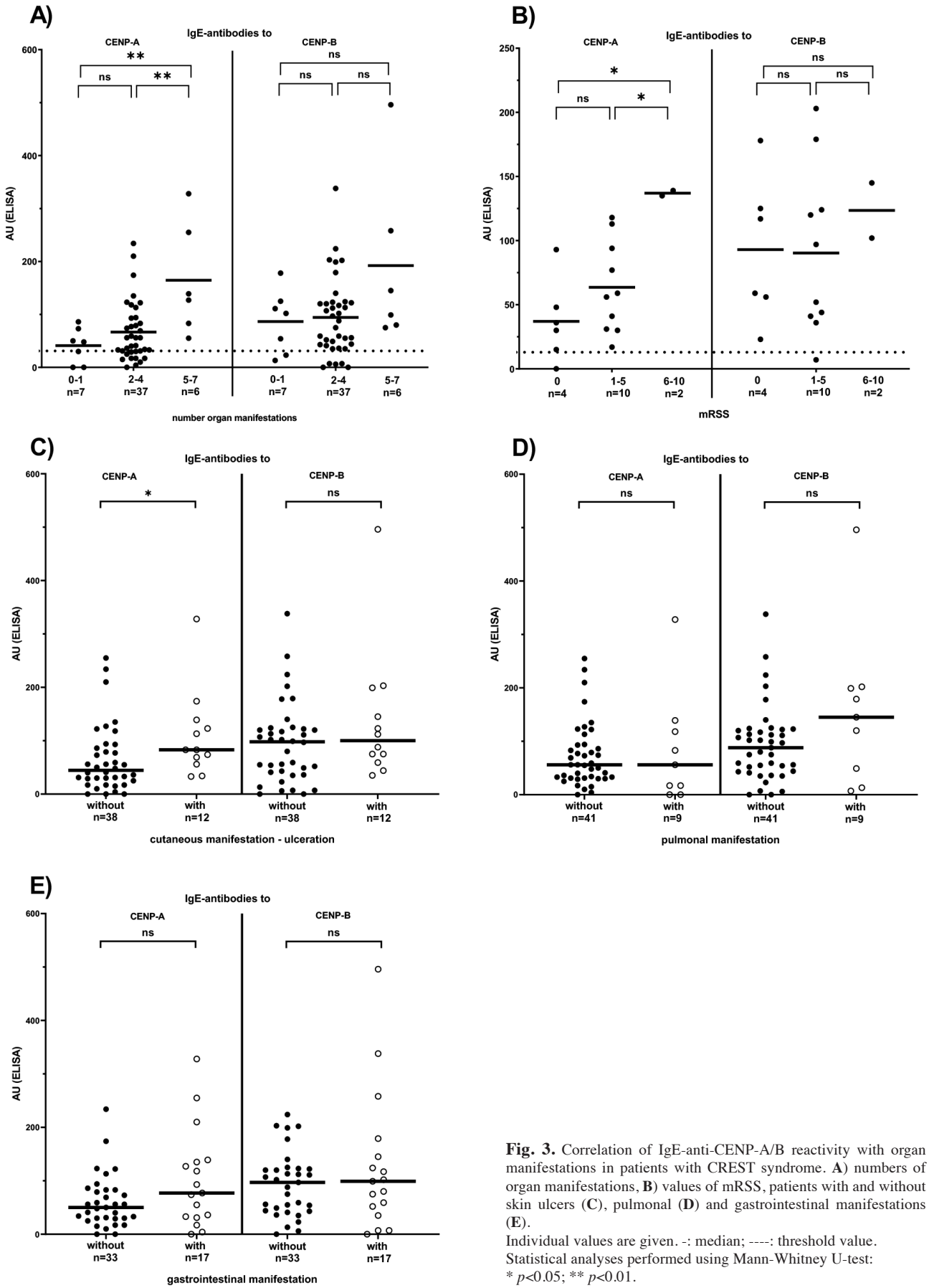

Fig. 3. Correlation of IgE-anti-CENP-A/B reactivity with organ manifestations in patients with CREST syndrome. **A**) numbers of organ manifestations, **B**) values of mRSS, patients with and without skin ulcers (**C**), pulmonal (**D**) and gastrointestinal manifestations (**E**).
Individual values are given. -: median; ----: threshold value. Statistical analyses performed using Mann-Whitney U-test: * $p<0.05$; ** $p<0.01$.

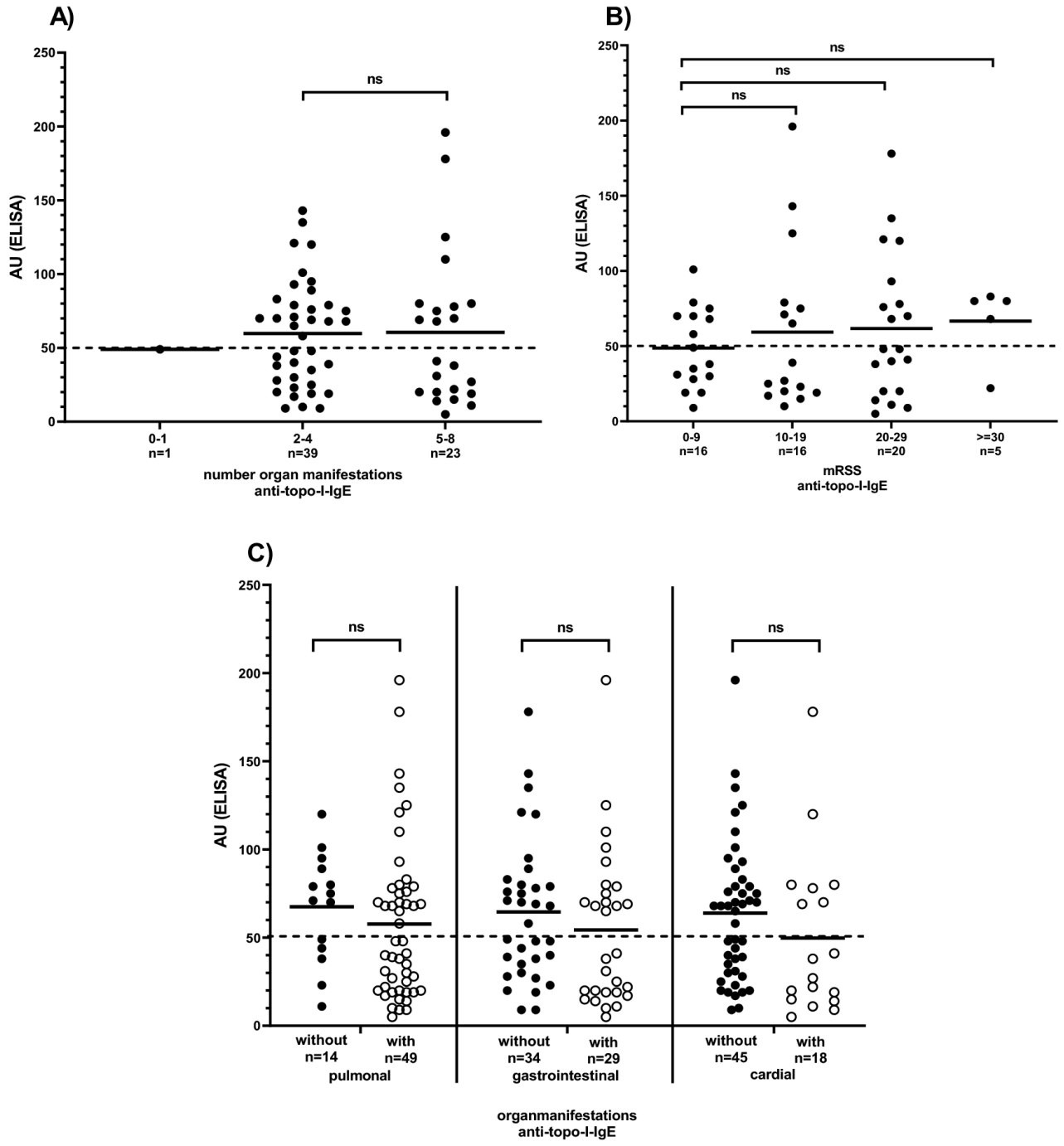

Fig. 4. Correlation of IgE-anti-topo-I reactivity with organ manifestations in patients with SSc (dcSSc and lcSSc).
A) Numbers of organ manifestation, **B**) values of mRSS, and **C**) patients with and without pulmonal, gastrointestinal, and heart manifestations.
Individual values are given. -: median; ----: threshold value.
No statistically significant differences were found.

and CREST syndrome and investigates their clinical relevance. The antibodies were detected by a newly developed in-house ELISA using recombinant antigens with native conformation which have been described to have a higher performance than extracted purified antigens in the current literature and are characterised by a consistent quality and high purity (21). As for the IgG-antibodies, anti-CENP-A/B antibodies of the IgE-type were characteristic for CREST syndrome and IgE-anti-topo-I antibodies for SSc. Of the SSc patients, 56% had anti-topo-I antibodies of the IgE-type; of the patients with CREST syndrome 67% had anti-CENP-A and even 78% anti-CENP-B antibodies of the IgE-type. In most instances, they were associated with the respective IgG-antibodies, and there was a strong correlation between IgG- and IgE-antibody reactivity.

Moreover, IgE-anti-CENP-A antibodies correlated with cutaneous manifestations such as mRSS and cutaneous ulcers as well as number of organs

involved in the disease while for anti-CENP-B- and anti-topo-I antibodies of the IgE-type no significant clinical associations were observed. Interestingly, anti-CENP-A/B IgE-antibodies seem to decrease during therapy with steroids or immunosuppressive drugs while the IgE-anti-topo-I antibodies were not influenced by this kind of therapy. However, there was some evidence that they may decrease after autologous stem cell transplantation.

Remarkably, IgE-antibody reactivity was in general much lower than the IgG-reactivity which may be due to the low levels of total IgE-immunoglobulins in patients' sera. Therefore, for the demonstration of autoantibodies of the IgE-type patients' sera had to be used at rather high concentrations, namely a dilution of 1:2 which is in marked contrast to the demonstration of antibodies of the IgG type where patients' sera have to be diluted 1:1,000 to obtain specific results (19). Nevertheless, despite this high serum concentration no non-specific binding was observed, and the specificity of this assay was proven by the absence of IgE-antibodies to topo-I and CENP-A/B in patients with other collagen disorders than SSc or CREST syndrome. Patients with Sjogren syndrome or SLE showing anti-topo-I or anti-CENP-A/B antibodies of the IgE-type had also IgG-antibodies and may suffer from an overlap syndrome. In each assay strongly and moderately positive as well as negative sera were applied as standard sera, resulting in highly reproducible and reliable results.

As already stated, anti-topo-I and anti-CENP-A/B antibodies of the IgE-type have not yet been described in the literature in patients with SSc or CREST syndrome. There is only one report showing the presence of anti-nucleolar IgE-antibodies in 56% of 16 patients with SSc and Raynaud's phenomenon (7). Moreover, it was shown that IgG- and IgM-antibodies to IgE immunoglobulins occur in SSc (22). In contrast, there are still quite a few studies investigating the presence of different other ANA of the IgE-type in connective tissue diseases such as SLE (9, 10, 11) or MCTD (12). The clinical relevance of these IgE-antibodies remained, however, unclear. An association between anti-SSA antibodies of the IgE-type and foetal loss in patients with Sjögren's syndrome and SLE was reported by Sekigawa *et al*. (13), and the presence of anti-dsDNA antibodies of the IgE-type in SLE patients seems to correlate with increased disease activity and nephritis (9).

In SSc, an association of anti-topo-I antibodies of the IgG-type with increased disease activity, interstitial lung disease, digital ulceration, and heart manifestations has been described by others and us (1, 23-25) and was also confirmed in the present study (data not shown). However, correlating anti-topo-I antibodies of the IgE-type with different clinical manifestations including number of organ manifestation or mRSS, we did not observe a significant association. In contrast, in CREST syndrome higher IgE-reactivity of anti-CENP-A antibodies of the IgE-type correlated with higher mRSS, more organ manifestations and presence of cutaneous ulcerations while a correlation between IgG-anti-CENP antibody reactivity and SSc has not been reported in the literature (26-28) and was also not observed in our patients (data not shown).

The role of IgE autoimmunity is still unclear. It has been shown that in SLE immune complexes consisting of dsDNA-IgE may affect dendritic cells by inducing maturation, antigen presentation, cellular migration and secretion of proinflammatory cytokines and may trigger DC-dependent B cell responses (10). Moreover, several studies suggest that dominance of T helper type 2 (Th2) cell dominance is a key immunological feature of SSc that directly and indirectly promotes fibrosis (29-32). Th2 cells support activation of eosinophils and B cells producing IgE immunoglobulins. In this respect it is of interest that SSc patients with skin ulcers showed significantly increased eosinophil counts compared with those without (20), and that eosinophils play an important role in the development of fibrosis in SSc (33, 34). However, in the present series we did not find a correlation between eosinophil counts and presence of ulcers or IgE-anti-CENP-A/B reactivity which may be due to selection criteria or low number of patients.

There are several limitations to our study. Thus, for distinct clinical manifestations number of patients was rather low, which may affect the statistical power of the analysis. Moreover, it is important to be aware that the prevalence of an antibody depends on the definition of cut off values. In our studies we chose the mean values of absorption plus threefold standard deviation determined in a group of autoantibody negative individuals. Using mean values plus twice the standard deviation might increase the sensitivity but leads to a loss of specificity. However, we could confirm the reliability of our threshold values also by ROC curves (data not shown). Furthermore, it would be of interest to analyse the reactivity of IgE-autoantibodies also in longitudinal studies during the course of the disease in order to see, if they are influenced by the appearance of distinct clinical manifestations, therapeutic regimes or environmental factors.

In summary, we have shown that anti-topo-I and anti-CENP-A- and -B antibodies in SSc and CREST syndrome are not only of the IgG- but also of the IgE-type. For the anti-CENP-A – but not the anti-CENP-B- and anti-topo-I – IgE-antibodies we found some association with disease activity and clinical manifestations. Since they were associated in most instances with the IgG antibodies they seem not to contribute to the serological diagnosis of the disease. But nevertheless, they may help to improve our understanding of the pathogenesis and immunological mechanisms in SSc.

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