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Anti-C1q Autoantibodies Are Frequently Detected in Patients With Systemic Sclerosis Associated With Pulmonary Fibrosis

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Title: Anti-C1q autoantibodies are frequently detected in patients with systemic sclerosis associated with pulmonary fibrosis

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Bulleted statements:

What's already known about this topic?

- In patients with systemic sclerosis (SSc) many autoantibodies are detected
- Some autoantibodies in SSc are likely to be involved in the pathogenesis of the disease
- Studies have reported complement activation in SSc
- Studies investigating antibodies against C1q, frequently detected in systemic lupus erythematosus, have not been performed in SSc

What does this study add?

- Anti-C1q antibodies are frequently detected in patients with SSc
- Anti-C1q antibody level is the most important risk factor for the presence of lung fibrosis

Abbreviations: CEN, centromere; CIC, circulating immune complexes; dc, diffuse cutaneous; lc, limited cutaneous; NC, normal control; RA, rheumatoid arthritis; RNA pol III, RNA polymerase III; Scl-70, scleroderma 70; SSc, systemic sclerosis; SLE, systemic lupus erythematosus; SjS, Sjogren's syndrome;

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Summary

Background: Anti-C1q autoantibodies (autoAbs) are associated with systemic lupus erythematosus (SLE) but their presence in other rheumatic diseases has not been adequately investigated.

Objective: We aim to assessanti-C1q autoAbs and circulating immune complexes (CICs) in systemic sclerosis (SSc).

Methods: One hundred twenty four patients with SSc (106 females; median age 59.4 years, range 25-81.4; 75(60.5%) with limited cutaneous SSc[lcSSc], and 49(39.5%) with diffuse cutaneous SSc[dcSSc]), were studied. Twenty-five patients with Sjögren's syndrome (SjS), 29 with rheumatoid arthritis (RA), and 38 patients with systemic lupus erythematosus (SLE) and 53 healthy controls (NC) were also included. ELISAs with high and low salt buffers the former allowing IgGFc binding to C1q, the latter not allowing IgGFc binding and anti-C1q Ab binding to C1q were used to measure anti-C1q Abs and CICs.

*Results:*Anti-C1q Abs were present in20/124 (16.1%) SSc patients [5 had high levels (>80 RU/mL) and 10 patients (50%) had moderate levels (40-80 RU/mL)] compared to 1/25 (4%) SjS, 1/29 (3.4%) RA patients (p<0.05, for all) and 3/53 (5.7%) NCs (p<0.01). Anti-C1q Abs were detected in 13/38 (34.2%) SLE patients. Anti-C1q Abs were more frequent in male than

female SSc patients (*p*=0.005); this association remaining after multivariate regression analysis . Anti-C1q Ab level was the first most important factor in predicting the presence of pulmonary fibrosis and the second in predicting pulmonary arterial hypertension. Fourteen SSc patients (11.3%) had CICs.

*Conclusions:*Anti-C1q autoAbs were frequently detected in patients with SSc and their high levels predict the co-occurrence of pulmonary fibrosis or pulmonary arterial hypertension.

Introduction

Systemic sclerosis (SSc)is characterized by extensive fibrosis, microvasculopathy including vasospastic episodes (Raynaud's phenomenon, RP)and fibrointimal proliferation, and the presence of many autoantibodies (autoAbs). The pathogenesis of the disease is incompletely understood [1, 2]. Microvasculopathy, as detected by nailfoldcapillaroscopy, and autoAbsappear years before skin fibrosis. Many autoAbs are detected in SSc, including disease-specific autoAbs, such as anti-topoisomerase I (anti-Topo I, formerly anti-Scl70) and anticentromere (anti-CEN)Abs which are independent predictors for the progression of RP to SSc[3-5]. Many autoAbs in SSc are likely to be pathogenic, since they promote inflammation and fibrosis[6, 7]. These include anti-endothelial cell [8, 9], anti-fibroblast [10], anti-matrix metalloproteinase-3 [11], anti-fibrillin-1 [12], anti-angiotensin II type 1 receptor [13], and anti-platelet-derived growth factor receptor antibodies[14, 15]. The presence of many autoAbsmight suggest activation of the classical pathway of complement. Indeed, studies in the eighties reported complement activation in SSc, as suggested by elevated plasma levels of complement fragment C3d and C4d[16]. Activated complement (C5b-9)and complement receptor for C5a (C5aR) were detected in the microvasculature of skin biopsies from patients with SSc[17].Also, reduced function of complement(C1,C4,C3) was detected in SSc[18], and reduced serum levels of C3 and/or C4 was detected in 14% of patients with SSc and were associated with vasculitis and myositis[19].

Anti-C1q autoAbsare frequently detected in systemic lupus erythematosus (SLE) and are strongly associated with lupus nephritis[20-24]. Two SLE studies with small numbers of SSc patients used as disease controls showed either a small frequency and increase[20]or no increased frequency[25] of anti-C1q autoAbs in SSc. It should be mentioned that the methodology of anti-C1q Ab detection faces a technical problem to distinguish between IgGFc (of immune complexes) binding to C1q and anti-C1 Ab binding to C1q. To clarify these observations and issues,we assessed the clinical significance of anti-C1q autoAbs in a large cohort of SSc patients.

Material and Methods

Patients and Controls

One hundred and twenty four patients with SSc (106 females; median age 59.4 years, range 25-81.4; 75 (60.5%) with limited cutaneous SSc[lcSSc], and 49 (39.5%) with diffuse cutaneous SSc[dcSSc]), were studied. All patients fulfilled the American College of Rheumatology criteria for SSc, and attended the Out-patient Systemic Sclerosis Clinic of the Department of Rheumatology and Clinical Immunology, at the University General Hospital of Larissa, in central Greece[4, 26, 27]. Demographic, laboratory and clinical data were collected for all patients (Table 1). Pulmonary arterial hypertension (PAH)was confirmed by right heart catheterization (RHC) and pulmonary fibrosis was diagnosed with high resolution CTscan. Conventional treatment regimens included low-dose steroids (<7.5 mg/day) plus azathioprine or methotrexate. All SSc patients had anti-nuclear antibodies by indirect immunofluorescence. Twenty five patients with Sjögren's syndrome (SjS), 29 with rheumatoid arthritis (RA), and 38 patients with systemic lupus erythematosus (SLE) served as disease controls, and 53healthy individualsused as normal controls (NCs). A written informed consent was obtained by all patients and NCs.

The protocol was approved by the Local Ethical Committee of the University General Hospital of Larissa, University of Thessaly, Larissa, Greece and all procedures were in accordance with the revised Declaration of Helsinki.

SSc-related AutoAb testing

All SSc sera are tested at diagnosis for ANA by indirect immunofluorescence, and for anti-centromere, anti-Topo I and anti-RNA polymerase III (RNA pol III) Abs. To ensure consistency, aliquots stored at -80 °C were tested at the same time for anti-C1q Abs by an ELISA and for anti-Topo I, anti-CEN and anti-RNA pol III Abs by an SSc profile line immunoassay (Euroimmun, Lubeck, Germany)[4]. This profile testing would enable correlation analysis between anti-C1q Abs and SSc-specific antibodies and was performed in 51SScpatients.

Anti-C1q Ab testing by ELISA

Anti-C1q antibodies were measured by enzyme-linked immunosorbent assay (ELISA) (QUANTA Lite® IgG anti-C1q, INOVA Diagnostics, Inc. San Diego, CA, USA). Testing was performed according to the manufacturer's instructions. Briefly,serum samples were diluted 1:10 with high ionic strength buffer that does not allow IgGFc (of circulating immune complexes, CIC) binding to C1q of plates, and 100 μLof diluted serum was incubated for 30 min at 20 °C.Then, microplate wells were washed (x3) and incubated for 30min at 20 °C with 100 μL of horseradish peroxidase-conjugated anti-human IgG. The plates were washed (x3) and incubated for 30 min in the dark with 3,3',5,5'tetramethylbenzidine (TMB) chromogen. The reaction was terminatedwith stop solution (0.344M sulfuric acid) after 15 minutes. The absorbance of the samples was read using a microplate reader (BIOTEK, Winnoski, VT, USA) at a wavelength of 450 nm/620 nm.Tests with >20 Relative Units (RU/mL)were

considered positive; 20-40 RU weakly positive; 40-80 RU moderately positive; and >80 RU strongly positive, according to the manufacturer's interpretation of results.

Measurement of circulating immune complexes (CICs) by ELISA

A second ELISAwas used to measure circulating immune complexes (CICs). Microwell plates pre-coated with purified human C1q(as above) were used (Inova). This ELISA with low salt concentration permits IgGFc (of CICs) binding, as well as anti-C1q Ab binding to C1q of the microplates. CIC-positivity was defined by subtracting high-salt ELISA serum sample positivity from low-salt ELISA positivity.

Corresponding data were presented in the form of scatter plots or Ab reactivity in patients and controls and VENN diagram, a set of circles each representing the total number of patients positive for an autoantibody, to illustrate the relationship between anti-C1q Abs and the three major SSc-related autoAbs tested for diagnostic purposes (anti-CEN, anti-Scl-70 and anti-RNA III pol autoAb)

Statistical analysis

The required sample size was estimatedusing the statistical software G-power for ANCOVA (Fixed effects, main effects and Interactions). Parametric and non-parametric tests were used for our study sample description, as appropriate. Univariate and multivariate regression analysis was used to study the effect of a battery of parameters on the levels of anti-C1q IgG levels.In anticipation for clinical and statistical interactions among the variables under study, the multivariate model was reduced to the most logically robust. To determine whether the manufacturer’s cut off for anti-C1q antibody levels could be used, we also assessed the cut-off for the anti-C1q IgG assay by plotting a receiver operator characteristic (ROC) curve using the test results of the patients with SSc versus normal or disease controls. A 95% CI has been calculated for the AUC. Finally, we used the GINI index from random forest

analysis to predict the importance of anti-C1q Abs among a bundle of variables in predicting pulmonary fibrosis and pulmonary arterial hypertension. Random forest analyses were implemented using the "randomForest" package with R. *P*-values smaller than or equal to 0.05 were considered significant. The statistical calculations were performed with Graph PadPrism Software 5 and R.

Results

Anti-C1q Ab testing

We performed a sample size estimation using G-power analysis for the multivariate model. Our study required 122 patients (a error probability: 0.05, power;1-β error probability:0.8 and degrees of freedom:3) (supplementary data). To this end, we obtained serum samples from 124 patients with SSc enrolled in our study. To assess whether the cut off value of 20 RU/mL set by the manufacturer applies to our SSc cohort, we performed ROC curve analyses (Fig 1). For a specificity (*vs* NC) set to 1 (95% CI, 0.89 to 1), cut off value had to be determined at 32.5 RU/mL (sensitivity 0.24, 95% CI, 0.15 to 0.36), while for a pre-determined specificity of 0.97 (95% CI, 0.83 to 1, LR, 7.42) the cut off value for anti-C1q positivity had to be set to 31.3 RU/ml. Taking into account that anti-C1q Ab positivity has been previously reported to be present in 3-7% of NC, we considered that the desired specificity had to be set to > 0.93 (Figure 1). According to our ROC analyses a 0.94 specificity (95% CI, 0.79 to 1, LR: 5.02) required a cut off value > 19.45 RU/mL. Thus, we concluded that the cut off value for anti-C1q Ab, set at 20 RU from the manufacturer, could also apply to our cohort.

Overall, anti-C1q Abs were present in 20/124 (16.1%) SSc patients compared to 1/25 (4%) SjS patients, 1/29 (3.4%) RA patients (p<0.05, for all) and 3/53 (5.7%) NCs (p<0.01). The frequency of anti-C1q Abs in patients with SLE was within the reported range (13/38, 34.2%). Amongst the 20 anti-C1q Ab-positive SSc patients, 5 (25%) had high levels (>80 RU/mL), and 10 patients (50%) had moderate levels (40-80 RU/mL).

Figure 3 shows a VENN diagram depicting anti-C1q Ab positivity and the three most specific SSc autoAbs (Anti-Topo, anti-CEN and anti-RNA pol III). Anti-Topo I, anti-CEN and anti-RNA pol III autoAbs did not differ between anti-C1q Ab-positive and anti-C1q Ab-negative patients (Fig. 3 and Table 1).

Clinical significance of anti-C1q Abs

Anti-C1q Abs were more frequent in male than female SSc patients (7/18, 38.9% *vs* 13/106, 12.3%, p=0.005) (Table 1 and 2, Supplementary Fig 1). The anti-C1q positivity was also more frequent in dcSSc than in lcSSc (12/49, 25% vs 8/75, 10.7%, p=0.049). The presence of anti-C1q Abs was associated with pulmonary fibrosis (p=0.023, Table 1). No other association was found between the presence of anti-C1q Abs and other clinical features (digital ulcers, PAH, arthritis, serositis, gastrointestinal involvement, telangiectasia, scleroderma renal crisis, and calcinosis). However, after performing a multiple regression analysis, only the effect of the gender persisted (Supplementary Table 2 and Supplementary Fig 1 and 2). Prediction analysis showed that the Anti-C1q Ab level is the first most important factor in predicting the presence of pulmonary fibrosis and the second important factor in predicting pulmonary arterial hypertension (Fig 4).

Prevalence and clinical significance of CICs

CICsare frequently found in patients with SLE but also in other autoimmune rheumatic diseases, including RA. CICs were calculated from the low salt buffer ELISA that allows,apart from anti-C1q Ab binding, IgGFc (of CICs) binding to C1q of the microplate wells. Overall, 34 (27.4%) SSc patients exhibited positivity with the low salt buffer ELISA, and therefore, by subtracting 20 patients with anti-C1q Abs, 14 SSc patients(11.3%) were positive for CICs. No association was found between CICs and demographic, clinical (digital ulcers, PAH, pulmonary fibrosis, arthritis, serositis, GI involvement, telangiectasias, scleroderma renal crisis, and calcinosis) or immunological (SSc autoAbs, such as anti-Topo I, antI-CENP and anti-RNA pol III autoAbs) features (Table 2).

Discussion

The present study found that anti-C1q Abs are frequently detectedin patients with SSc. There are two reports of anti-C1q Abs testing in SLE, which included patients with SSc. One SLE study with 55 SSc patients reported a small increase but low frequency of anti-C1q Abs in SSc compared to NCs (5.5% vs 2.1%) [20] and a second SLE study with 20 SSc patients reported no difference in the frequency of anti-C1q autoAbs between SSc and NCs (10% *vs* 10%) [25].The increased prevalence of anti-C1q Abs in our cohort may relate to the enhanced sensitivity of the assay. As mentioned, the methodology of anti-C1q Ab detection faces a technical problemto distinguish between IgGFc (of CICs) binding and anti-C1q Ab binding to C1q of the ELISA plate. This problem has been solved by the use of high salt concentration in the assay(1 M NaCl) that disrupts the binding of IgGFc to C1q[28].

C1q is part of the C1 complex (the other parts being serine proteases C1r and C1s), a critical component of classical complement pathway of activation. The activation of the classical complement pathway is initiated by binding of globular head of C1q to Fc region of

IgG complexed to antigen. Anti-C1q abs bind to collagen-like tail of C1q[29].The anti-C1q ELISA by INOVA we utilized, has a high ionic strength buffer to disrupt the binding of IgGFc (of immune complexes) to C1q[28, 30] and thus detects only anti-C1q Abs. We also utilized an ELISA with low ionic strength buffer to detect both anti-C1q Abs plus IgGFc-C1q binding (circulating immune complexes). By using both assays we could measure anti-C1q Abs as well as CICs and assess associations of anti-C1q Abs but also associations of CICs.

The diagnostic utility of anti-C1q autoAbs is relatively low since only 5 out of 26 patients without SSc-specific anti-CEN, anti-Topo I or anti-RNA pol III autoAbs have anti-C1q autoAbs. Nevertheless, our study shows that serum IgGanti-C1q autoAbs are detected in a significant percentage of SSc patients; in fact, anti-C1q Abs are equally prevalent with anti-RNA pol III Abs, which have so far been considered the third most prevalent autoAb in patients with SSc. Furthermore, the presence of anti-C1q Abs appears to be more prevalent in male patients with SSc. The numbers of male patients in this study is small as expected, since the proportion of male patients in SSc large cohorts and registries is 12%-16%[31, 32]. Yet, the association of anti-C1 Abs with male sex stands in multivariate analysis. The pathophysiological significance of this association is not clear at present. Of relevance, the prognostic value of anti-C1q levels is highlighted by prediction analysis showing that the anti-C1q Ab level is the first most important factor in predicting the co-occurrence of pulmonary fibrosis and the second important factor in predicting pulmonary arterial hypertension. Prospective studies must address the significance of this finding, and if such studies are proved to be correct, the potential of anti-C1q to be used as clinical biomarker must be studied in greater detail.

It is logical to assume that alterations of the classical pathway of complement relates to microvascular injury. Microvascular damage is an early and critical component of SSc. Endothelial cell apoptosis in skin biopsies was detected very early in avian scleroderma, and in the early inflammatory stage of human SSc[33, 34] and was caused by anti-endothelial cell antibodies[33, 35]. Microvascular clinical manifestations of SSc are serious and include digital ulcers, resorption of terminal phalanges of digits, PAH, and scleroderma renal crisis. It may also contribute to organ fibrosis through hypoxia. Male sex is associated with microvascular features in SSc. For instance, male sex is a risk factor for progression of early PAH in SSc[36], and for early mortality in SSc[37, 38] with PAH being the most common cause of death[37]. Male sex is also a risk factor for mortality in patients at risk for PAH[39]. Nevertheless, the relation of male sex with anti-C1q Abs and PAH should be assessed in larger studies.

However, C1q has other functions independent of complement activation. For instance, C1q binds to apoptotic cells and increases their clearance by phagocytes (opsonisation)[40-42]. Furthermore, it exerts immunosuppressive effects, as C1q-bound apoptotic cells suppressed dendritic cell- and macrophage-mediated Th1 and Th17 proliferation[43]. Therefore, the presence of anti-C1q autoAbs may be related to autoimmunity[44, 45]and thus may be involved in the pathogenesis of SSc. As mentioned, anti-C1q abs bind to collagen-like tail of C1q and this likely impairs the removal of apoptotic cells[29]. Indeed, anti-C1q Abs from lupus nephritis patients bind to C1q on early apoptotic cells and significantly reduce phagocytosis of early apoptotic cells by macrophages[45]. This suggests that a defective clearance of apoptotic cells may serve as a source of autoantigens in SSc. Anti-C1q autoAbs from lupus nephritis patients also inhibit the deposition of C3c on immune complexes(IC)-C1q(opsonisation of ICs) and the binding of opsonized ICs to red blood cells[45], thus suggesting another source of persisted autoantigens in SSc.Of relevance, we assessed the presence of CICs in our SSc cohort, and 14 SSc patients (11.3%) tested positive for CICs.Another means by which anti-C1q autoAbs are involved in autoimmune disease is through neutrophil extracellular

traps(NETs). NETs, formed by neutrophils on exposure to pathogens, contain nuclear chromatin and granule enzymes, to fight pathogens but may also serve as a source of autoantigens and promote autoimmunity. C1q binds to NETs(NET opsonisation) thus facilitating their clearance[46]. Abs binding to NETs from sera of SLE patients inhibit NETs degradation and this impairment correlates with lupus nephritis[47]. However, it is not known if NET formation is increased in SSc.

In summary, our study found increased frequency of anti-C1q autoAbs and CICs (both products of plasma cells) in patients with SSc and particularly in male patients. The presence of increased levels of anti-C1q Abs is a predictive factor for the presence of lung fibrosis and pulmonary arterial hypertension. Their involvement in the pathogenesis of SSc needs further assessments.[48-51]However, their clinical significance needs to be further assessed in multicentre studies with well-defined SSc patients.

Disclosure of interest

Professor Lazaros I. Sakkas received travel expenses from ELPEN Ltd, Greece, to attend the EULAR 2018 meeting in Amsterdam, travel expenses from Actelion Pharmaceutical, Greece, to attend the 2018 World Scleroderma Conference in Bordeaux, travel expenses from Pfizer, Greece, to attend the 11th International Congress on Autoimmunity in Lisbon. He also received speaker's honoraria from Sanofi and Actelion.

Associate Professor Dimitrios P. Bogdanos received travel expenses from ELPEN Ltd, Greece, to attend EULAR, Amsterdam, 2018 travel expenses from Roche Hellas and ELPEN Ltd, for participation in local meetings within Greece. He also received speaker's honoraria from Inova Diagnostics, San Diego, Ca, USA (within 2018) and Menarini Ltd, Italy (within 2017).

Dr Theodora Simopoulou received travel expenses from ACTELION PHARMACEUTICAL, Greece, to attend World Scleroderma Conference, Bordeaux, France and Genesis pharma S.A , Greece, to attend EULAR 2018 in Amsterdam. She also received speaker's honoraria from UCB Pharma, Elpen, and Janssen Pharmaceutica.

All other authors declare that they have no competing interest.

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Legends to the Figures

Figure 1.ROC for anti-C1q antibodies in SSc patients versus normal controls or SSc patients versus pathological controls

Figure 2. Vertical Scatter plot illustration of anti-C1q antibody responses expressed as Relative units (RU) in patients with SSc (n=124), Sjögren's syndrome (n=25), rheumatoid arthritis (n=29), systemic lupus erythematosus (n=39) and in 53 normal controls (NC). Solid lines in each group express mean RU±SEM. Tests were considered positive when values were >20 Relative Units (RU); weak positive in between 20-40 RU; moderately positive in between 40-80 RU; and strongly positive >80 RU, according to the manufacturer's interpretation of results.

Figure 3.VENN diagram showing concurrent IgG anti-C1q, anti-CEN, anti-Scl-70 and anti-RNA III pol autoantibody reactivities in 51 patients with SSc patients.

Figure 4. Figure. Random forest variable importance plot highlighting the role of anti-C1q in (A) predicting pulmonary fibrosis (PF) and (B) pulmonary arterial hypertension (PAH). As illustrated anti-C1q iAb level s the first most important factor in predicting the development of PF and the second (after age) important factor in predicting PAH.

Supplementary Figures

Supplementary Figure 1. The effect of gender in the anti-C1q antibody levels.

Supplementary Figure 2. The effect of disease phenotype extension of the anti-C1q antibody levels.

Table 1. Clinical and immunological associations between anti-C1q antibody positive and anti-C1q antibody negative patients with systemic sclerosis (SSc): Univariate analysis.

	Total SSc patients (n=124)	**Anti-C1q (+)SSc(n=20)**	**Anti-C1q (-)SSc(n=104)**	***p* value**
Epidemiological				
Mean age, years (±SD)	53.8 (23)	54.5.8 (24.3)	43.3.8 (30.2)	**ns**
Females, n (%)/Males, n (%)	106 (85.5%)/18(14.5%)	13(65)/7 (35%)	44 (89.4%)/11 (10.6%)	**0.005**
Type of SSc				
Limited/limited cutaneous, n (%)	75 (60.5)	8 (40)	67 (64.4%)	**0.039**
Diffuse cutaneous, n (%)	49 (39.5)	12 (60)	37 (35.6%)	
Clinical features				
Digital Ulcers, n (%)	52 (41.9)	10 (50)	42 (40.4)	0.425
Pulmonary fibrosis, n (%)	41 (33.1)	11 (55)	30 (28.8)	**0.023**
Pulmonary Arterial Hypertension (n,%)	19(15.3)	4 (20)	15(14.4)	0.508
Arthritis (n,%)	23 (18.5)	5 (25)	18(17.3)	0.455
Serositis (n,%)	8 (6.5)	1 (5)	7 (6.7)	0.766
Telangiectasia (n,%)	68 (54.8)	12 (60)	56 (53.8)	0.643
Calcinosis (n,%)	17 (13.7)	2 (10)	15 (14.4)	0.588
GI involvement (no, upper, upper and low, n,%)	49 (40.3)/ 69 (55.6)/ 5(4)	1 (5)/7(35)/12(60)	4 (3.9)/62 (59.6)/38 (36.5)	0.112
Renal Crisis	2 (1.6)	0 (0)	2 (1.9)	>0.99
SSc-specific autoAbs*				
Anti-centromere, n (%)	35 (28.2)	7(35)	28(26.9)	0.478
Anti-Topo I, n (%)	53 (42.7)	8 (40)	45 (43.3)	0.760
Anti-RNA III pol, n (%)	18 (14.5)	1 (5)	17 (16.3)	0.301

Table 2. Clinical and immunological associations between CIC-C1q positive and CIC-C1q negative patients with systemic sclerosis (SSc).

	Total SSc patients (n=124)	**CIC-C1q (+)SSc(n=34)**	**CIC-C1q (-)SSc(n=90)**	***p* value**
Epidemiological				
Mean age, years (±SD)	53.8 (23)	47.4 (28.3)	54.7 (25.4)	0.197
Females, n (%)/Males, n (%)	106 (85.5%)/18(14.5%)	25(73.5)/9 (26.5)	81 (90)/9 (10%)	**0.002**
Type of SSc				
Limited/limited cutaneous, n (%)	75 (60.5)	16(47)	59 (64.5)	**0.056**
Diffuse cutaneous, n (%)	49 (39.5)	18 (53)	31 (35.5)	
Clinical features				
Ulcers, n (%)	52 (41.9)	17 (50)	17 (38.8)	0.263
Pulmonary fibrosis, n (%)	41 (33.1)	16 (39)	18 (20)	**0.046**
Pulmonary Arterial Hypertension (n,%)	19(15.3)	7 (36.8)	12(13.3)	0.317
Arthritis (n,%)	23 (18.5)	7 (20.6)	16 (17.8)	0.782
Serositis (n,%)	8 (6.5)	1 (2.9)	7 (7.8)	0.322
Telangiectasia (n,%)	68 (54.8)	22 (64.7)	46 (51.1)	0.194
Calcinosis (n,%)	17 (13.7)	4(11.8)	13 (14.4)	0.683
GI involvement (no, upper, upper and low, n,%)	49 (40.3)/ 69 (55.6)/ 5(4)	16(47)/17(50)/1(3)	33 (36.7)/52 (57.8)/4 (4.5)	0.585
SSc-specific autoAbs*				
Anti-centromere, n (%)	35 (28.2)	11(32.3)	24(26.7)	0.554
Anti-Topo I, n (%)	53 (42.7)	15 (44.1)	38 (42.2)	0.760
Anti-RNA III pol, n (%)	18 (14.5)	2 (5.9)	16 (17.8)	0.314