

# Hypocomplementemia in Systemic Sclerosis — Clinical and Serological Correlations

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**ABSTRACT.** *Objective.* Although complement fixation is not commonly thought to be part of the pathogenesis of systemic sclerosis (SSc), hypocomplementemia has been associated with SSc. We hypothesized that hypocomplementemia in SSc might indicate the presence of overlap disease. We investigated if SSc patients with hypocomplementemia had more features of overlap disease than those with normal complement levels.

*Methods.* Study subjects consisted of those enrolled in the Canadian Scleroderma Research Group Registry. Patients were divided into 2 groups: those with normal complement levels (normal C3 and C4) and those with hypocomplementemia (low C3 or C4). Evidence of overlap disease was defined as physician reports of other specific rheumatic conditions. Autoantibodies were assayed. Differences in rates of concomitant diseases and in antibody profiles were compared between groups.

*Results.* Our study included 321 patients (88% women, mean age  $56 \pm 13$  yrs, mean disease duration  $11 \pm 9$  yrs). Of these, 276 (86%) had normal complements and 45 (14%) had hypocomplementemia. Patients with hypocomplementemia were significantly more likely to have physician-reported inflammatory myositis (27% vs 12%;  $p < 0.008$ ) and vasculitis (11% vs 2%;  $p < 0.011$ ) than those with normal complement. There was also a trend toward more antichromatin antibodies (18% vs 9%;  $p = 0.051$ ) in patients with hypocomplementemia compared to normals.

*Conclusion.* Hypocomplementemia may identify a particular subgroup of SSc patients who have overlap disease. (First Release Oct 15 2007; J Rheumatol 2007;34:2218–23)

## Key Indexing Terms:

SCLERODERMA      COMPLEMENTS      AUTOIMMUNE DISEASE      CLASSIFICATION

Systemic sclerosis (SSc) is a multisystem disorder characterized by a disturbance in fibroblast function, microvascular disease, and immune system activation, culminating in fibrosis of skin and internal organs<sup>1</sup>. It is associated with significant morbidity, including disfiguring skin thickening, finger ulcers, joint contractures, pulmonary hypertension, interstitial lung disease, chronic diarrhea, and renal failure. Functional disability is considerable<sup>2</sup>, depressive symptoms and major depression are common<sup>3–5</sup>, and quality of life is impaired<sup>6,7</sup>.

The pathogenesis of SSc is complex. Abnormalities in 3 cell types, namely fibroblasts, endothelial cells, and cells of the immune system, particularly T and B lymphocytes, have been described. These account for the characteristic triad of

pathologic changes seen in SSc: cutaneous and visceral fibrosis, obliteration of the lumen of the microvasculature, and immune dysfunction characterized by the production of autoantibodies (some very specific for the disease). This is accompanied by mononuclear cell infiltration of affected tissues and dysregulation of lymphokine and growth factor production<sup>8</sup>. Although an explanation of the pathogenesis of SSc unifying these 3 cell lines is still lacking, a recent study identified stimulatory autoantibodies to the platelet-derived growth factor receptor on fibroblasts, thereby activating collagen gene expression<sup>9</sup>. This finding links at least 2 of the cardinal pathogenic features of SSc by suggesting that autoantibodies could be one of the factors that sustain the profibrotic phenotype of fibroblasts.

Hypocomplementemia has on occasion been described in association with SSc. In the work that led to the American College of Rheumatology (ACR) classification criteria for SSc, low C3 had been identified as a “promising” laboratory variable to be studied for possible inclusion in the criteria<sup>10</sup>. In the end, though, it was not retained. Subsequently, a study of 34 patients with SSc and 20 healthy controls reported normal complement levels but significantly higher levels of complement fragments, namely C3d, C4d, and Ba, in the SSc patients compared to the controls, suggesting that measurable amounts of complement activation occur in SSc<sup>11</sup>. Most

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recently, the European Scleroderma Study Group published a preliminary disease activity index for SSc that includes hypocomplementemia as one of the 10 variables used to compute a disease activity score<sup>12</sup>.

We hypothesized that, rather than being a primary component of disease pathogenesis, hypocomplementemia in SSc might be a marker of overlap disease. We therefore undertook to determine if SSc patients with hypocomplementemia had more features of overlap disease than those with normal complement levels.

## MATERIALS AND METHODS

This was a cross-sectional study of a cohort of patients with SSc.

**Study subjects and sources of data.** Subjects consisted of those enrolled in the Canadian Scleroderma Research Group (CSRG) Registry. Patients in this registry are recruited from the practices of rheumatologists across Canada. They must have a diagnosis of SSc made by the referring rheumatologist, be  $\geq 18$  years of age, be fluent in English or French, and be likely to be compliant with study procedures and visits. A patient with evidence of overlap disease could be included provided the rheumatologist was also convinced that the patient had SSc. Patients were included who had a baseline visit between September 2004 and August 2006 and who had data on complement levels entered into the database as of November 2006.

Patients in the CSRG Registry undergo an extensive standardized evaluation, including a yearly history and physical examination by a physician and laboratory testing. Among other things, physicians are asked to report the presence of inflammatory arthritis, arterial or venous thrombosis, inflammatory myositis, vasculitis, or overlap disease with another definite rheumatic disease including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), polymyositis, dermatomyositis, Sjögren's syndrome, or mixed connective tissue disease. Specific data to confirm these diagnoses are not elicited. Physicians also complete global assessments of disease activity, severity, and damage using an 11-point numerical rating scale. The numerical rating scale is simple to complete and score and has been shown to be as reliable and responsive as visual analog scales to measure disease activity and function in ankylosing spondylitis<sup>13</sup> and more reliable to assess pain in patients with RA<sup>14</sup>. Patients also complete a series of self-administered questionnaires to determine symptoms and measure function, depression, pain, and health related quality of life. These questionnaires include the following:

1. Scleroderma-Health Assessment Questionnaire (S-HAQ), which consists of the HAQ-Disability Index (DI)<sup>15</sup> and scales to measure the severity of symptoms specific for SSc, namely Raynaud's phenomenon, digital ulcers, gastrointestinal symptoms, lung symptoms (shortness of breath), and overall disease severity in the past week<sup>2</sup>. Unlike the visual analog scales originally used for the S-HAQ, the assessments in this study were also made using an 11-point numerical rating scale;
2. Center for Epidemiologic Studies–Depression Scale (CES-D)<sup>16,17</sup>;
3. Short-Form McGill Pain Questionnaire<sup>18–20</sup>; and
4. The Medical Outcomes Study Short-Form 36 (SF-36)<sup>21,22</sup>, for which scores can be summarized into a Physical Component Summary (PCS) score and a Mental Component Summary (MCS) score. Scores range from 0 (worst) to 100 (best).

Disease activity was measured using the Valentini Scleroderma Disease Activity Index<sup>12,23</sup>. It consists of 10 weighted variables: total skin score  $> 14$ , sclerodema, digital necrosis, arthritis, total lung capacity  $< 80\%$ , erythrocyte sedimentation rate  $> 30$  mm/h, hypocomplementemia, and change in cardiopulmonary, skin and vascular symptoms in the past month. The final score ranges from 0 (no activity) to 10 (very active). Disease severity was measured using a modified Medsger Scleroderma Disease Severity Scale<sup>24,25</sup>. The original scale assessed disease severity in 9 organ systems, namely, general health, peripheral vascular, skin, joint/tendon, muscle, gastrointestinal tract, lungs, heart, and kidneys. Each organ is scored separately from 0 to 4 depend-

ing on whether there is no, mild, moderate, severe, or endstage involvement. For the purposes of this study, the worst category was scored for each system and results of any investigation not requested by the physician, therefore missing, were considered "normal." Scoring methods for general, peripheral vascular, skin, joint/tendon, lung, and kidney systems were identical to those proposed in the original scale. Some adaptations were made to the other organ systems. In order to assign a score for the skeletal muscle system, physicians were asked to rate patients' muscle strength in 5 different areas of the body (neck flexors, as well as upper and lower proximal extremities, right and left) using the British Medical Research Council scale<sup>26</sup>. A severity score was then assigned depending on the total number of 5s, 4s, 3s, 2s, 1s, and 0s for a given patient. The HAQ-DI was used to assess the patient's use of ambulation aids needed to assign the worst severity level for the skeletal muscle system (i.e., level 4, endstage). To score the gastrointestinal system, in addition to an abnormal esophagram, abnormal esophageal manometry, or abnormal small bowel series, patients reporting difficulty swallowing, acid taste in their mouth, choking at night, burning sensation, feeling of being full shortly after eating, or taking gastroprotective or promotility agents were also given a score of 1 for mild. In addition to malabsorption syndrome and episodes of pseudo-obstruction, patients with an abnormal hydrogen breath test were given a score of 3 for severe. To score the heart system, electrocardiogram results, percentage left ventricular ejection fraction values, presence of conduction abnormalities, distended neck veins, and arrhythmia diagnosed by a physician were used.

Serum was collected on all patients recruited in the CSRG Registry and sent to a central laboratory at the Advanced Diagnostics Laboratory, University of Calgary, Calgary, Alberta. Aliquots of sera are stored at  $-70^{\circ}\text{C}$  until needed. Antinuclear and anticentromere antibodies were assessed by indirect immunofluorescence on HEp-2000 substrate (ImmunoConcepts Inc., Sacramento, CA, USA) and antibodies to extractable cell antigens [chromatin, Sm, U1-RNP, ribosomal P, Jo-1, topoisomerase I (topo-I), SSA/Ro, SSB-La] by addressable laser bead immunoassay using a commercial kit (QuantaPlex SLE Profile 8; Inova Diagnostics, San Diego, CA, USA).

**Outcome measures.** C3 and C4 levels were measured in the clinical laboratories of the local recruiting centers on sera obtained at the baseline visit. Since normal values for complement levels vary slightly depending on individual assays, patients were defined as having normal or low values according to local laboratory ranges. Patients were divided into 2 groups, those with normal complement levels, defined as having normal C3 and C4, and those with hypocomplementemia, defined as having low C3 or C4.

Evidence of overlap disease was defined as physician reports of inflammatory arthritis, arterial or venous thrombosis, inflammatory myositis, vasculitis, overlap disease with another definite rheumatic disease, SLE, RA, polymyositis, dermatomyositis, Sjögren's syndrome, or mixed connective tissue disease. Autoantibodies were assayed as described above.

**Statistical analysis.** Differences in rates of overlap features and in antibodies between patients with normal levels of both C3 and C4 and those with hypocomplementemia were compared using Fisher's exact test if any cell in the  $2 \times 2$  comparison was less than 10 or otherwise using chi-squares. P values less than 0.05 were considered statistically significant. No adjustment for multiple testing was done because our objectives were exploratory and such an adjustment would have considerably reduced the possibility of identifying clinically significant findings. Thus, the p value serves as a relative measure of potential clinical interest rather than as a mechanism for performing formal tests of hypotheses. Similar reasons not to correct for multiple comparisons have been discussed previously<sup>27</sup>. Nevertheless, the reader should be aware that some of the significant findings reported here might be the result of type I error. All statistical analyses were performed with SPSS v. 13.

## RESULTS

This study included 321 patients from the CSRG Registry who had data on C3 and C4 levels: 88% women, mean age  $56 \pm 13$  years, mean disease duration since onset of first non-

Raynaud's manifestation of SSc  $11 \pm 9$  years, 89% Caucasian, and 88% met ACR classification criteria for SSc. Of these, 276 (86%) had normal complements and 45 (14%) had hypocomplementemia.

Patients with normal complements and hypocomplementemia were generally similar in terms of baseline (Table 1) and disease characteristics (Table 2). However, patients with hypocomplementemia were consistently more likely to have concomitant physician-reported diagnoses of other rheumatic diseases compared to those with normal complements (Table 3). In particular, the rates of inflammatory myositis (27% vs 12%;  $p < 0.008$ ) and vasculitis (11% vs 2%;  $p < 0.011$ ) were significantly higher in those with hypocomplementemia compared to those with normal complements, and there was a trend toward more diagnosis of definite overlap (24% vs 13%;  $p = 0.059$ ) in the former as well. There was also a trend toward more antichromatin antibodies (18% vs 9%;  $p = 0.051$ ) in patients with hypocomplementemia compared to

normals. Of note, there were significantly more patients with anti-topo-I antibodies in those with hypocomplementemia compared to those with normal complements (27% vs 16%;  $p = 0.045$ ), but not with antibodies to centromere, Sm, U1-RNP, Jo-1, ribosomal P, or SSA/Ro or SSB/La.

## DISCUSSION

In this cohort of 321 patients with SSc, we found that patients with hypocomplementemia were more likely to have features of overlap disease than those with normal levels of complement. Specifically, inflammatory myositis and vasculitis were more frequent in those with hypocomplementemia, and there was a trend toward an increase in patients classified as having overlap disease. Therefore, consistent with our hypothesis, hypocomplementemia in SSc may, at least in part, reflect the coexistence of another connective tissue disease. On the other hand, some studies have documented the presence of circulating autoantibodies and immunoglobulin deposits in tissues of

*Table 1.* Baseline characteristics of a cohort of patients with systemic sclerosis (SSc) as a whole and according to complement status. Data are percentages or mean (SD).

	Whole Cohort, n = 321	Normal Complements, n = 276	Hypocomplementemia, n = 45
Women, %	88	87	96
Race/ethnic group, %*			
Caucasian	89	89	84
Native American	7	6	13
Others	21	21	22
Fulfill ACR criteria for SSc, %	88	88	84
Age, yrs	56 (13)	57 (12)	51 (16)
Disease duration, yrs**	11 (9)	11 (9)	12 (10)
Physical global assessments (range 0–10)			
Disease severity	2.7 (2.3)	2.7 (2.4)	2.6 (1.9)
Disease activity	2.2 (2.0)	2.1 (2.0)	2.5 (2.0)
Disease damage	3.2 (2.4)	3.2 (2.4)	3.0 (2.3)

\* Numbers sum to more than 100% because patients are allowed to choose more than one category. \*\* Since onset of first non-Raynaud's disease manifestation.

*Table 2.* Comparison of selected disease characteristics according to complement status. All numbers represent means (standard deviations), except presence of fingertip ulcers and tendon friction rubs, which represent percentages.

	Whole Cohort, n = 321	Normal Complements, n = 276	Hypocomplementemia, n = 45
Modified Rodnan skin score (range 0–51)	11 (10)	11 (11)	10 (10)
Presence of fingertip ulcers, %	9	8	18
No. of fingertip ulcers	6 (5)	6 (5)	7 (5)
Presence of tendon friction rubs, %	9	9	11
Forced vital capacity (% predicted)	93 (42)	94 (44)	86 (20)
DLC0 (% predicted)	75 (33)	74 (32)	81 (39)
Pulmonary artery pressure, mm Hg	40 (18)	40 (18)	40 (15)
No. of gastrointestinal symptoms	4 (3)	4 (3)	4 (3)
Serum creatinine, $\mu\text{mol/l}$	82 (41)	81 (36)	85 (64)
C-reactive protein, mg/dl	6 (7)	6 (7)	4 (7)
Erythrocyte sedimentation rate, mm/h	24 (23)	24 (22)	25 (29)

Table 3. Comparison of rates of overlap disease and antibody status according to complement status.

	Normal Complements, n = 276 N (%)	Hypocomplementemia, n = 45 N (%)	p
Inflammatory arthritis	99 (36)	20 (44)	0.269
Arterial or venous thrombosis	7 (3)	3 (7)	0.155
Inflammatory myositis	33 (12)	12 (27)	0.008
Vasculitis	6 (2)	5 (11)	0.011
Overlap	37 (13)	11 (24)	0.059
Rheumatoid arthritis	11 (4)	4 (9)	0.243
Systemic lupus erythematosus	6 (2)	3 (7)	0.120
Polymyositis	3 (1)	2 (4)	0.148
Dermatomyositis	1 (0.4)	0	1.000
Sjögren's syndrome	17 (6)	3 (7)	1.000
Mixed connective tissue disease	4 (1)	2 (4)	0.210
Antinuclear antibody-positive	198 (72)	31 (69)	1.000
Anticentromere pattern	61 (22)	9 (20)	1.000
Topoisomerase-I	44 (16)	12 (27)	0.045
Sm	8 (3)	2 (4)	0.627
U1-RNP	19 (7)	6 (13)	0.119
SSA/Ro	49 (18)	8 (18)	0.829
SSB/La	6 (2)	3 (7)	0.103
Jo-1	6 (2)	1 (2)	1.000
Chromatin	24 (9)	8 (18)	0.051
Ribosomal P	6 (2)	2 (4)	0.289

patients with SSc and, although definitive *in vivo* confirmation of complement activation in SSc is lacking, it remains a possibility in some patients<sup>11</sup>.

We also found a trend toward patients with hypocomplementemia having more antichromatin antibodies. Since these antibodies are thought to be relatively specific for SLE<sup>28,29</sup>, finding antichromatin antibodies in patients with hypocomplementemia also supports the hypothesis of some degree of overlap with other diseases. The significance of finding more anti-topo-I antibodies in patients with hypocomplementemia is uncertain. Anti-topo-I antibodies have also been shown to be specific for SSc<sup>30-32</sup>. Nevertheless, these antibodies have on occasion been reported in patients with SLE<sup>33</sup>. In one study, anti-topo-I antibodies were found in 32 (25%) of 128 randomly selected patients with SLE<sup>34</sup>. Thus, consistent with our hypothesis, there may be a subset of patients with SSc and anti-topo-I antibodies who have overlap disease.

Although some studies have suggested that complement-fixing autoantibodies distinguish idiopathic SLE from drug-induced lupus<sup>35</sup>, other studies have shown that even anticentromere antibodies have the capacity to fix complement<sup>36</sup>. Further, the finding that hypocomplementemia was associated with antichromatin antibodies is of interest in the context of studies that showed that patients with SSc bearing antihistone antibodies often had a poorer clinical outcome than patients with anticentromere antibodies<sup>37</sup>. Similarly, patients with anti-topo-I also tend to have more severe disease and a poorer outcome<sup>38</sup> than, for example, patients with antibodies to centromere or anti-PM/Scl antibodies<sup>39</sup>.

It is interesting that in other large SSc cohorts recently described, 10%–27% were reported as having overlap disease<sup>40,41</sup>. Our finding that 14% of patients with SSc in this cohort had hypocomplementemia is consistent with these reports.

The significance of finding a marker of overlap disease in SSc is 3-fold. First, from a clinical point of view, most studies of immunosuppressive drugs in SSc have failed to show substantial clinical benefits<sup>42-45</sup>. However, hypocomplementemia may provide a biomarker for patients who may require consideration for such therapy. Second, from a research point of view, the high prevalence of features of overlap disease in patients with SSc supports the emerging concept of shared autoimmunity, where certain genes predispose to autoimmune diseases in general. This would explain the well recognized conundrum of overlap features among patients who have a specific disease phenotype as well as familial associations of seemingly different autoimmune disease<sup>46</sup>. Research to determine how gene-environment interactions lead to the expression of specific clinical disease phenotypes continues and may, in the future, dramatically change the way we classify autoimmune diseases. Third, several groups are in the process of updating classification criteria and are attempting to develop measures of disease activity for SSc<sup>12,47</sup>. One reason for having classification criteria and measures of disease activity is to identify appropriate patients for inclusion in clinical trials and to measure outcome in those trials. From an efficacy point of view, in those trials, it may be advisable to study patients with only SSc. On the other hand, the results of a

study excluding patients with features of overlap may not be generalizable to all patients with SSc, as in our study where features of overlap were noted in up to 14% of all patients with SSc. A compromise may be to include all patients with SSc but to analyze outcomes by subgroups of patients with and without features of overlap. Thus, researchers interested in classification criteria and measures of disease activity need to be aware that some SSc patients may have overlap and may or may not be included in clinical trials, and they need to decide in advance whether or not to include these patients in the cohorts used to develop and validate any new instrument.

It is noteworthy that only 88% of our cohort, and 84% of those with hypocomplementemia, met the ACR classification criteria for SSc<sup>10</sup> (Table 1). However, it is well known that the criteria lack sensitivity and many experts have noted that they exclude 10% or more of patients with SSc<sup>48</sup>. For this reason, we recruited patients in our cohort based not on the criteria but on the diagnosis of a rheumatologist. By the same token, we are confident that the patients in the cohort have SSc, although a certain proportion do not meet ACR criteria, and that our findings are not due to misdiagnosis of the underlying disease.

There are some limitations to our study. First, although patients with overlap are not excluded from the CSRG Registry, we are primarily interested in SSc. Thus, we collect extensive clinical and laboratory data relevant to SSc but not necessarily to other autoimmune diseases. We therefore do not have all the data necessary to confirm that patients who were identified as having overlap disease indeed had other diseases. Nevertheless, the presence of overlap diseases was confirmed by a certified rheumatologist and is thus likely to be accurate.

Second, we measured only intact C3 and C4 levels rather than degradation products of complement activation. However, intact components may be an insensitive measure of complement activation because, as acute-phase proteins, their degradation may be compensated by accelerated production<sup>11</sup>. Some have therefore suggested that measurement of complement degradation products may be more sensitive markers of complement activation<sup>11</sup>. Nevertheless, the lack of sensitivity in measuring intact C3 and C4 would have resulted in underestimating the number of patients with hypocomplementemia. Thus, our findings can be considered conservative estimates.

Third, hypocomplementemia might represent a genetically determined effect rather than increased utilization or turnover of complement as occurs in immune complex-mediated conditions. This has not yet been studied in SSc and may need to be addressed in the future.

Finally, we failed to identify an increase in frequency of other autoimmune diseases, such as Sjögren's syndrome, that are also associated with overlap with SSc and hypocomplementemia. However, the overall frequency of Sjögren's in our cohort was 6%, and this failure may be due to a lack of power.

The strength of our study lies in the large sample size. This is the largest study to date to describe complement levels in patients with SSc. In addition, our cohort spans Canada. Thus,

our patients are geographically and culturally diverse. This adds to the generalizability of our findings.

Hypocomplementemia in SSc may, at least in part, reflect the coexistence of overlap disease. This finding may have implications for clinical decision-making for patients with SSc and both basic science and outcomes research in the field of SSc.

## APPENDIX

Canadian Scleroderma Research Group Investigators: M. Abu-Hakima, Calgary, Alberta; P. Docherty, Moncton, New Brunswick; J. Dunne, Vancouver, British Columbia; N. Jones, Edmonton, Alberta; N. Khalidi, Hamilton, Ontario; S. LeClercq, Calgary, Alberta; J. Markland, Saskatoon, Saskatchewan; J-P. Mathieu, Montreal, Quebec; J. Pope, London, Ontario; P. Rahman, St. John's, Newfoundland; D. Robinson, Winnipeg, Manitoba; D. Smith, Ottawa, Ontario; E. Sutton, Halifax, Nova Scotia.

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