Systemic sclerosis–rheumatoid arthritis overlap syndrome: a unique combination of features suggests a distinct genetic, serological and clinical entity

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Objective. To determine the genetic, clinical and serological characteristics of systemic sclerosis (SSc)-rheumatoid arthritis (RA) overlap syndrome.

Methods. Clinical manifestations and immunolaboratory features of 22 SSc-RA patients were assessed. The HLA-DR genotype of the 22 SSc-R A patients determined by SSP-PCR was compared with that of 38 SSc patients, 100 RA patients and 50 healthy controls.

Results. All overlap patients fulfilled the American College of Rheumatology (ACR) criteria for SSc and RA. Five of the 22 patients (23%) had diffuse cutaneous SSc (dcSSc) and 17 patients (77%) had limited cutaneous SSc (lcSSc). Antinuclear antibody, anti-Scl70, IgM rheumatoid factor and anti-CCP antibody positivity were detected in 22 (100%), 5 (23%), 16 (73%) and 18 patients (82%), respectively. Seventeen patients (77%) had pulmonary fibrosis, 12 (55%) had oesophageal dismotility, 11 (50%) had cardiac and five (23%) had renal involvement. Hand joint destruction was observed in 18 patients (82%). Significantly increased frequencies of HLA-DR3 (36% vs 5%), HLA-DR7 (9% vs 4%), HLA-DR11 (36% vs 7%) and HLA-DRw53 (23% vs 5%) were observed in SSc-RA compared with RA patients (P<0.05). Allele frequencies of the ‘shared epitope’ (HLA-DR1 and -DR4) were significantly increased in SSc-RA (32% and 27%, respectively) and RA patients (46% and 31%, respectively) in comparison with SSc patients (10.5% and 16%, respectively) or healthy controls (16% and 14%, respectively) (P<0.05).

Conclusions. To date this is the largest SSc-RA overlap cohort. Genetics, clinical and immunolaboratory features suggest a mixed phenotype. Our data suggest that SSc-RA overlap syndrome may be a distinct genetic, immunological and clinical entity.

KEY WORDS: Rheumatoid arthritis, Systemic sclerosis, Overlap syndrome, HLA-DR.

Introduction
Well-established connective tissue diseases generally develop without overlap features. On the other hand, features of two or more systemic autoimmune diseases may develop in some patients, where each component fulfils the diagnostic criteria of the American College of Rheumatology (ACR). Thus in some cases of systemic sclerosis (SSc), the disease may overlap with other organ-specific or systemic autoimmune diseases, such as rheumatoid arthritis (RA), polymyositis, systemic lupus erythematosus and others [1, 2]. The overlap between SSc and RA is rather uncommon and to date only few cases of SSc-RA overlap have been reported [3-9]. Jinnin et al. [9] described nine SSc patients with concomitant RA. Horiki et al. [6] reported five SSc-RA cases. All patients had diffuse cutaneous form of SSc (dcSSc) and anti-topoisomerase I antibodies with synovitis and erosions of small joints typical of RA. Zimmermann et al. [7] presented three patients with longstanding RA and consecutive evolution of limited cutaneous SSc (lcSSc) with the presence of autoantibodies typical of both diseases. Recently, Ingegnoli et al. [10] reported an association between anti-cyclic citrullinated peptide (CCP) antibody positivity and the presence of arthritis in SSc patients.

Supposing that overlap syndrome of SSc and RA may be a distinct clinical and genetic entity, we assessed 22 Hungarian patients with SSc-RA overlap. Clinical and immunological features, as well as their HLA-DR genotypes of these overlap patients are analysed. Furthermore, genetics of SSc-RA overlap patients are compared with established SSc, RA patients and healthy control subjects.

Patients and methods
Clinical and serological assessment
RA-SSc overlap patients were selected from 477 SSc patients collected during the last 16 yrs in Debrecen and Pécs. Altogether 22 SSc-RA overlap patients (16 females and 6 males) were recruited from Hungary. Their mean age was 53.0±14.6 yrs (range: 34–72 yrs). All patients fulfilled the ACR criteria for both SSc and RA [11, 12]. Clinical features including pulmonary, cardiac, oesophageal manifestations and others were analysed. Pulmonary fibrosis was defined as present by radiographic findings and pulmonary function tests. Oesophageal involvement was assessed by barium radiography of the oesophagus. Cardiac involvement indicated by relaxation abnormalities, conduction disturbances or right ventricular hypertrophy was assessed by ECG, as well as two-dimensional and Doppler echocardiography. Immunological analyses included tests for antinuclear antibodies (ANA), anti-centromere antibody (ACA), anti-topoisomerase I antibody (anti-Scl70), IgM rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibody. ANA and ACA were determined by indirect immunofluorescence on HEP-2 cells. ANA positivity was assessed at 1:40 dilution, Anti-Scl70, anti-CCP and anti-U1RNP were determined in all patients by ELISA (Cogen Diagnostics, UK). IgM RF was assessed by nephelometry. The normal values for IgM RF and anti-CCP were <50 U/ml and <25 U/ml, respectively. HLA-DR genotyping described later in detail was also performed in these SSc-RA overlap patients, as
well as in 38 other patients with SSc (31 females and 7 males; mean age: 49.3 ± 9.8 yrs) and 100 other patients with RA (75 females and 25 males; mean age: 46.7 ± 7.9 yrs). All patients fulfilled the ACR criteria for SSc [11] and RA [12], respectively. None of the 22 overlap patients had a family history of autoimmune diseases. Regarding therapy, the 17 patients having pulmonary fibrosis (Table 1) received cyclophosphamid before, but none of them at the time of this study. RA symptoms were generally treated with methotrexate and sulphasalazine, but patients with pulmonary fibrosis did not receive methotrexate. All 22 overlap patients received an ACE inhibitor, a calcium channel blocker and pentoxifylline.

Blood was also obtained from 50 matched healthy controls (44 females and 6 males; mean age: 51.6 ± 11.4 yrs). They were either hospital employees or visitors who were unrelated to the patients. The control group underwent the same procedures as the patient groups.

A written consent was obtained from each individual according to the declaration of Helsinki. In addition, this study has been approved by the local ethics committee of our university.

Polymerase chain reaction with sequence specific primers (PCR-SSP)

Genomic DNA was isolated from buffy coats of EDTA anticoagulated blood using QIAamp Blood Mini Kit (QIAGEN GmbH, Germany) according to the instructions of the manufacturer. Polymerase chain reaction (PCR)-based HLA-DRB typing (DRB1*01-DRB1*16) was performed (Ollerup SSP, Genovision, Norway). All samples were processed according to the instructions of the manufacturer using recombinant Taq DNA polymerase (Invitrogen, Brazil). HLA-DRB genotypes were determined on the basis of the PCR pattern obtained using 2% agarose gel electrophoresis. DNA bands were detected using Alpha Imager MultiImage Light Cabinet (Alpha Innotech Corporation, San Leandro, CA, USA).

Statistical analysis

Statistical analysis was carried out using Fisher’s exact test and chi-squared test. Pearson’s correlation was also performed when needed. P-values <0.05 were considered as statistically significant.

Results

Clinical description of patients with SSc-RA overlap syndrome

A summary of the clinical and immunological features of the 22 patients with SSc-RA overlap syndrome is shown in Tables 1 and 2. Currently, 477 SSc patients are undergoing follow-ups at our institutions in Debrecen (Eastern Hungary) and Pécs (Southwestern Hungary). Among these 477 patients, 22 (4.6%) also developed RA. These 22 overlap patients, as described earlier, fulfilled the ACR criteria for both SSc and RA. Five of the 22 patients (23%) had dcSSc and 17 patients (77%) had lcSSc. The diagnosis of RA followed that of SSc in 19 patients (86.4%) and preceded that of SSc in three patients (13.6%). None of the overlap patients had exacerbation of the primary disease at the time of onset of their secondary disease.

ANA positivity was detected in all SSc-RA patients (100%), anti-topoisomerase I antibody positivity in 5 (22.7%), IgM RF positivity in 16 (72.7%), anti-CCP positivity in 18 (81.8%) and ACA positivity in only 2 (9.1%) SSc-RA patients. The mean ± s.d. values for IgM RF and anti-CCP were 81.8 ± 52.0 U/ml and 105.1 ± 111.3 U/ml, respectively. All overlap patients were negative for anti-U1RNP autoantibody and thus none of them had mixed connective tissue disease (MCTD).

Regarding organ manifestations, 17 of the 22 patients (77.3%) had pulmonary fibrosis, 12 (54.5%) had esophageal oesophageal dysmotility, while 11 (50%) had cardiac and five (22.7%) had renal involvement. Out of the 17 patients with pulmonary fibrosis, 13 (76.5%) had low diffusion capacity for CO (DLCO), 12 had restrictive (8 mild, 2 moderate and 2 severe) and three (2 mild, 1 moderate) had obstructive respiratory insufficiency. However, none of the patients had respiratory failure. Two out of the 22 overlap patients (9.1%) had pulmonary arterial hypertension (PAH). All patients with renal involvement (n = 5) had chronic renal failure; however, none of them had scleroderma renal crisis or renal hypertension. Three overlap patients with lcSSc (13.6%) fulfilled the criteria for CREST syndrome and three patients (13.6%) had secondary Sjögren’s syndrome.

Marked articular destruction and erosions were observed on the proximal interphalangeal (PIP), metacarpophalangeal (MCP), carpal joints or ulnar heads in 18 (81.8%) SSc-RA patients. No

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TABLE 1. Clinical features of 22 patients with SSc-RA overlap syndrome

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>SSc subtype</th>
<th>Age at the time of diagnosis (yr)</th>
<th>Raynaud’s phenomenon</th>
<th>Joint destruction and erosion</th>
<th>Pulmonary fibrosis</th>
<th>Renal</th>
<th>Cardiac</th>
<th>Oesopha-gal</th>
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<td>F</td>
<td>dcSSc</td>
<td>33</td>
<td>36</td>
<td>+ (+-ulcer)</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>lcSSc</td>
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<td>56</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>53</td>
<td>(+-ulcer)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>62</td>
<td>(+-ulcer)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>24</td>
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<td>+</td>
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<td>58</td>
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<td>+</td>
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<td>43</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<td>lcSSc</td>
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<td>51</td>
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<td>+</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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</table>

SSc: systemic sclerosis; dc: diffuse cutaneous; lc: limited cutaneous; RA: rheumatoid arthritis.
significant associations were found between any clinical and serological features (data not shown).

**HLA-DRB1 genotype of patients with SSc-RA**

The HLA-DRB1 genotypes of the 22 patients with SSc-RA overlap syndrome are shown in Table 3. We also compared HLA-DRB1 genotypes of the overlap patients with genotypes of SSc, RA patients and healthy subjects (Table 4). Allele frequencies of HLA-DRB1 and HLA-DR1 were significantly increased in SSc-RA overlap patients (36% and 36%, respectively) compared with RA patients (5% and 7%, respectively) or to healthy controls (18% and 20%, respectively) (P < 0.05). No such differences were observed between SSc-RA and SSc patients (Table 5). HLA-DR3 and HLA-DR11 frequency was also significantly higher in SSc patients (47% and 42%, respectively) compared with RA patients (5% and 7%, respectively) and to the control group (18% and 20%, respectively) (P < 0.05). On the other hand, HLA-DR1 and HLA-DR4, namely the 'shared epitope' genotypes, exerted significantly higher frequencies in SSc-RA (32% and 27%, respectively) and RA patients (46% and 31%, respectively) compared with either SSc patients (10.5% and 16%, respectively) or controls (16% and 14%, respectively) (P < 0.05). Thus, the allele frequencies of HLA-DR1 and HLA-DR4 were similar in SSc-RA overlap syndrome and in RA (Table 5).

**Discussion**

In the present study, the genetic, serological and clinical characteristics of SSc-RA overlap syndrome were assessed in the to-date largest cohort of 22 patients. The prevalence of SSc-RA overlap is 4.3% [8] to 5.2% [9] among SSc patients, which is
similar to the incidence (4.6%) found in our cohort. In addition, higher incidence of RA is found within SSc patients than in the general population [8, 9]. Thus, it is clinically important to check for the occurrence of RA in patients with established SSc.

The early diagnosis of RA is often difficult in patients with SSc because arthritis may be commonly present in both diseases. Symmetric polyarthritis and contractures of the joints are frequently observed in both RA and SSc, however, some of the clinical and pathological features of articular involvement are different in these disorders. Furthermore, radiographic joint destruction is usually less severe in SSc than in RA [13].

Regarding the clinical features of SSc-RA overlap patients, 19 of our 22 SSc-RA overlap patients described here had long-term SSc with various internal organ manifestations that was later complicated by the development of RA, usually 1–16 yrs after the onset of SSc. Only three patients developed RA prior to the diagnosis of SSc. Jinnin et al. [9] reported similar sequence pattern of the development of the two diseases. In that study, the incidence of SSc-RA overlap was assessed in 173 SSc patients. Among the patients, nine (5.2%) developed consecutive RA. The diagnosis of RA followed that of SSc in all nine patients. In contrast, Zimmermann et al. [7] reported three cases of SSc-RA overlap. All three patients had long-term erosive RA preceding the onset of lcSSc.

The clinical presentation of our SSc-RA patients showed a mixture of organ manifestations characteristic for SSc and RA. Erosive polyarthritis (82%), pulmonary fibrosis (77%), oesophageal involvement (55%) and cardiac manifestations (50%) were most commonly observed, while renal involvement occurred in 23% of the patients. Furthermore, 23% of patients had dcSSc and 77% had lcSSc. In the study of Horiki et al. [6], all five SSc-RA patients had dcSSc with severe seropositive polyarthritis and pulmonary fibrosis associated with anti-topoisomerase I seropositivity.

Regarding immunological analyses, all overlap patients were ANA positive, 23% of our patients showed anti-topoisomerase I and 9% exerted ACA seropositivity. Jinnin et al. [9] reported that all nine SSc-RA patients were ACA negative and they detected anti-topoisomerase I in 55.5% of their patients. As described earlier, all five patients of Horiki et al. [6] had anti-topoisomerase I seropositivity. The three patients described by Zimmermann et al. [7] had lcSSc associated with ACA positivity. Thus, our patients exert a unique clinico-serological pattern as the majority of them have lcSSc with anti-topoisomerase seropositivity in 23% of the patients but ACA positivity in only 9% of them. These differences may be due to the smaller number of SSc-RA patients in other cohorts or to the geographical heterogeneity of patients in various studies. In addition to the SSc-related autoantibodies, IgM RF positivity and anti-CCP positivity were observed in 73% and 82% of our patients, respectively. Ingegnoli et al. [10] reported an association between anti-CCP and erosive polyarthritis in SSc. These data indicate that SSc-RA overlap patients may be characterized by a distinct mixed serological pattern resembling both SSc and RA.

In the present study, we analysed the genetic background of SSc-RA overlap syndrome compared with SSc and RA patients, as well as to healthy controls. Significantly increased frequencies of HLA-DR3 and HLA-DR11 were observed in SSc-RA in comparison with RA patients and healthy subjects. In addition, allele frequencies of the HLA-DR1 and HLA-DR4 ‘shared epitope’ genes were significantly higher in SSc-RA and RA than in SSc or controls. Thus, SSc-RA overlap patients have HLA-DR3 and HLA-DR11 allele frequencies similar to SSc and HLA-DR1 and HLA-DR4 frequencies similar to RA. In various cohorts, SSc has been associated with HLA-DR3 and HLA-DR11, while HLA-DR1 and HLA-DR4 have been implicated in the pathogenesis of RA [14–20]. The association between susceptibility to RA and the ‘shared epitope’ has been well established [14–16]. Horiki et al. [6] reported an association of HLA-DR4 with dcSSc, severe seropositive polyarthritis, pulmonary fibrosis and anti-topoisomerase I antibody positivity in their five overlap patients. Regarding SSc-related HLA-DR genes, HLA-DR3, as well as anti-topoisomerase I autoantibodies have been associated with pulmonary fibrosis in SSc patients. HLA-DR3 and/or anti-topoisomerase I antibody positivity result in a 16.7-fold risk to develop pulmonary fibrosis in SSc [17]. In the study of Venneker et al. [18], HLA-DR3 and HLA-DR5 were associated with lcSSc and dcSSc, respectively. In our present study, we also detected the HLA-DR3 allele in one-third of SSc-RA patients and, as described earlier, the majority of our patients had lcSSc. In contrast, we did not test our patients for HLA-DR5. Loubière et al. [19] reported an increased frequency of HLA-DR11 in Caucasian women with SSc. In another study, pulmonary fibrosis in SSc has also been associated with HLA-DR11 [20]. Thus, our SSc-RA overlap patients carried both the SSc-associated HLA-DR3 and HLA-DR11 alleles, as well as the RA-related HLA-DR1 and HLA-DR4 alleles.

In summary, 4.6% of our SSc patients undergoing follow-ups at our institutions had SSc-RA overlap syndrome. The onset of SSc preceded that of RA in most cases. The majority of the patients had lcSSc with ANA, IgM RF and anti-CCP positivity in SSc and anti-topoisomerase I positivity in one-quarter of the patients. Regarding genetics, SSc-RA overlap patients carry both the SSc- and RA-associated HLA-DR alleles. These data support that SSc-RA overlap syndrome may be a distinct genetic, serological and clinical entity. As SSc-RA overlap patients in many ways differ from either RA or SSc patients, our data may have significant clinical relevance. A detailed clinical, radiological, laboratory and genetic assessment of either RA or SSc patients may reveal some overlap cases, which need special attention including individual treatment and follow-up.

The authors have declared no conflicts of interest.

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