Clinical and autoantibody profile in systemic sclerosis: baseline characteristics from a West Malaysian cohort

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Abstract

Aim: To evaluate the clinical and antibody profile of systemic sclerosis (SSc) in a Malaysian cohort.

Methods: Consecutive patients with SSc in University Malaya Medical Centre from March to November 2012 were included in this study. In addition to clinical characterization, all subjects underwent autoantibody testing using Euroline immunoblot assay. The association between clinical features and autoantibody profile was evaluated.

Results: There were 31, predominantly Chinese (45.2%), subjects. Limited cutaneous disease was the most common subtype (71%). Raynaud’s phenomenon was the most commonly observed feature (83.9%). Nine (29%) had esophageal dysmotility symptoms and 23 (74.2%), including all patients with diffuse SSc, had symptoms of gastro-esophageal reflux disease (GERD). Restrictive pattern on pulmonary function test and evidence of lung fibrosis were seen in more than 70% of patients. Echocardiographic evidence of pulmonary arterial hypertension was seen in 58.1%. Telangiectasia, calcinosis, digital ulcers, digital pulp loss or pitting were seen more commonly in the diffuse subtype. The two most prevalent autoantibodies were anti-Scl-70 and anti-Ro-52. The presence of anti-Scl-70 was significantly associated with restrictive lung disease (P = 0.05). Anti-Ro-52 was associated with control subjects with other autoimmune diseases (P = 0.043). The presence of anti-PM-Scl-75 was associated with overlap syndrome (P = 0.032). Patients with anticentromere antibodies were more likely to have vasculitic rash (P = 0.012).

Conclusion: In Malaysia, SSc most commonly affects the Chinese. Limited cutaneous is more common than diffuse subtype. Features of CREST (calcinosis, Raynaud disease, esophageal dysmotility, sclerodactyly, telangiectasia) are more commonly observed in the diffuse cutaneous subgroup. Anti-Scl-70 and anti-Ro-52 antibodies are promising biomarkers for pulmonary involvement in SSc.

Key words: autoantibodies, clinical profile, Malaysia, systemic sclerosis.
disease (GERD) and esophageal dysmotility, are frequent and can lead to reduced quality of life.

Antinuclear antibodies are detected in more than 96% of patients with SSc. A number of autoantibodies have been identified and are reported to be useful biomarkers associated with clinical phenotypes and survival. These autoantibodies have been established as strong predictors of disease outcome and pattern of organ complications. Classic examples include anti-Scl-70 antibodies and anti-centromere antibodies (ACA). The former increases the risk of diffuse skin involvement and lung fibrosis, whereas the latter is a predictor of limited cutaneous disease in the absence of pulmonary fibrosis. Anti-Scl-70, ACA and other useful markers, such as anti-Th/To, anti-fibrillarin and anti-RNA polymerase III, are able to predict the onset of disease before a clear clinical diagnosis can be established. Other autoantibodies, such as anti-fibrillarin, anti-Ku and anti-Ro-52, are not specific for SSc but are associated with SSc at varying levels of clinical significance.

The aim of this study was to evaluate the clinical profile of SSc and its association with autoantibodies in a Malaysian cohort.

MATERIALS AND METHODS

Study subjects and controls
The recruitment was done prospectively at a teaching hospital, University of Malaya Medical Centre (UMMC), between March and November 2012. The study was approved by the UMMC Medical Ethics Committee (IRB reference no: 907.12) and it conforms to the provisions of the World Medical Association’s Declaration of Helsinki. Thirty-four consecutive patients who fulfilled the 1980 American College of Rheumatology criteria for SSc were initially selected. However, only 31 patients had given their informed consent to participate in this study. Demographic and clinical details were obtained from a review of medical records, history taking and physical examination. Thrity-one age, gender and race-matched subjects were included as control subjects. The subjects in the control group consisted of 11 healthy persons and 20 patients with the following conditions: six systemic lupus erythematosus (SLE), five rheumatoid arthritis (RA), four Sjögren’s syndrome, two ankylosing spondylitis, one psoriatic arthritis, one inflammatory myositis and one mixed connective tissue disease.

Clinical assessment
All subjects with SSc underwent multi-disciplinary clinical evaluation, which included pulmonary function test (PFT), 6-min walk test (6MWT), transthoracic echocardiogram and electrocardiogram (ECG). The lungs were involved when PFT showed a restrictive lung disease pattern (forced vital capacity [FVC] of < 75%), and/or abnormal 6MWT, and/or evidence of pulmonary fibrosis on chest X-rays (CXR) or high-resolution computed tomography (HRCT) of the thorax. The CXR and HRCT films were assessed if they had been done within the last 12 months. PAH was suspected if pulmonary arterial systolic pressure (PASP) was ≥ 30 mmHg on echocardiography. Cardiac disease was recorded as present when structural abnormality was detected on echocardiography, conduction defects on ECG, or features of congestive heart failure. A comprehensive history was obtained, especially on cardiovascular, respiratory and upper gastrointestinal systems. The results of esophago-gastro-duodenoscopy (EGDS) were obtained retrospectively if the procedure had been done.

Autoantibody testing
The Euroline autoantibody test kit (Euroimmun AG, Lübeck, Germany) used in this study consists of 13 SSc-specific antigens, including Scl-70 (from bovine and rabbit thymus), centromere antigen subunits (CENP A, CENP B), PM-Scl-100, PM-Scl-75, Ku, Ro-52 (from baculovirus system), RNA Polymerase III subunits (RP11 and RP 155), fibrillarin (U3RNP), nucleolus-organizing regions (NOR)-90, Th/To (from Escherichia coli), and platelet-derived growth factor receptor (PDGFR: mammalian cells). The sera of all subjects and controls were tested by the Euroline immunoblot immunoglobulin G (IgG) technique. Initially, serum samples diluted at 1 : 100 were incubated with the test strips. In positive samples, the specific IgG antibodies have bound to the corresponding antigenic site. A second incubation was carried out to detect the fixed antibodies using enzyme-labelled anti-human IgG which displayed a color reaction. The reaction intensities were automatically graded by EurolineScan (Euroimmun AG, Lübeck, Germany), a computer software that presents the result as either strongly positive, positive, borderline positive or negative. Antibodies with borderline and weakly positive signal intensities were considered negative in this study.

Statistical analysis
The analyses were performed using SPSS version 19 (software package (IBM), Chicago, IL, USA). The data were presented as mean values (± SD), unless otherwise specified. Clinical associations with the SSc antibodies
were evaluated using chi-square, Fisher’s exact or Mann–Whitney U-tests, where appropriate. The level of significance was taken as $P \leq 0.05$.

RESULTS

The majority of SSc patients in this study were females (28, 90.3%). By race, they comprised 45.2% Chinese, 38.7% Malays and 16.1% Indians. Limited cutaneous disease (lcSSc) and diffuse cutaneous disease (dcSSc) were identified in 22 (71%) and nine (29%) patients, respectively. Overlap syndrome was seen in six (19.3%) patients, in which four out of six predominantly had lcSSc, while the remaining two patients had dcSSc. The disease duration ranged from 7 months to 31 years with a mean of 8.8 ($\pm$ 6.9) years. The lag time from onset of symptoms until diagnosis varied between 2 to 60 months with a mean duration of 14 ($\pm$ 16.7) months (Table 1).

Clinical features

The most frequent physical sign observed in this cohort was Raynaud’s phenomenon (26, 83.9%), followed by facial skin tightness (24, 77.4%) and sclerodactyly (24, 77.4%). A large proportion of subjects also had arthritis (21, 67.7%) and microstomia (23, 74.2%). The less common features were calcinosis (15, 48.4%), telangiectasia (12, 38.7%), digital ulcers (8, 25.8%), digital pitting or loss of digital pad substance (14, 45.2%) and vasculitic rash (5, 16.1%). Features such as telangiectasia, calcinosis, pitting, digital ulcers and digital pad loss were significantly more common in dcSSc than lcSSc (Table 2).

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>lcSSc</th>
<th>dcSSc</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raynaud's phenomenon</td>
<td>18 (81.8%)</td>
<td>8 (88.8%)</td>
<td>0.633</td>
</tr>
<tr>
<td>Telangiectasia</td>
<td>6 (27.2%)</td>
<td>6 (66.6%)</td>
<td>0.044*</td>
</tr>
<tr>
<td>Digital ulcers</td>
<td>3 (13.6%)</td>
<td>5 (55.5%)</td>
<td>0.017*</td>
</tr>
<tr>
<td>Digital pad loss or pitting</td>
<td>7 (31.8%)</td>
<td>7 (77.7%)</td>
<td>0.020*</td>
</tr>
<tr>
<td>Calcinosis</td>
<td>7 (31.8%)</td>
<td>8 (88.8%)</td>
<td>0.005*</td>
</tr>
<tr>
<td>Arthritis</td>
<td>16 (72.7%)</td>
<td>5 (55.5%)</td>
<td>0.774</td>
</tr>
<tr>
<td>Microstomia</td>
<td>16 (72.7%)</td>
<td>7 (77.7%)</td>
<td>0.784</td>
</tr>
<tr>
<td>Facial skin tightness</td>
<td>17 (77.2%)</td>
<td>7 (77.7%)</td>
<td>0.976</td>
</tr>
<tr>
<td>Sclerodactyly</td>
<td>16 (72.7%)</td>
<td>8 (88.8%)</td>
<td>0.337</td>
</tr>
<tr>
<td>Lung fibrosis</td>
<td>13 (68.1%)</td>
<td>7 (77.7%)</td>
<td>0.723</td>
</tr>
<tr>
<td>Pulmonary arterial hypertension</td>
<td>11 (50%)</td>
<td>7 (77.7%)</td>
<td>0.201</td>
</tr>
<tr>
<td>Esophageal dysmotility symptoms</td>
<td>6 (27.2%)</td>
<td>3 (33.3%)</td>
<td>0.740</td>
</tr>
<tr>
<td>Reflux (GERD) symptoms</td>
<td>14 (63.6%)</td>
<td>9 (100%)</td>
<td>0.039*</td>
</tr>
<tr>
<td>Heart conduction defects</td>
<td>2 (9.1%)</td>
<td>2 (22.2%)</td>
<td>0.591</td>
</tr>
</tbody>
</table>

lcSSc, limited cutaneous systemic sclerosis; dcSSc, diffuse cutaneous systemic sclerosis; GERD, gastro-esophageal reflux disease; *$P \leq 0.05$.

The symptoms suggestive of GERD were present in 23 patients (74.2%), while esophageal dysmotility was seen in nine patients (29%). The symptoms of GERD were moderate to severe, usually occurring once a week with some patients (9, 29%) suffering daily. A small number of patients also reported atypical chest pain (5, 16.1%) and epigastric pain (6, 19.3%). The presence of these upper gastrointestinal symptoms neither correlated with disease duration nor age at onset. We noted that the proportion of patients who suffered from GERD was significantly different between the SSc subtypes ($P = 0.039$); specifically, GERD symptoms were present in all patients with dcSSc compared to 60.9% in lcSSc. Five patients had previous EGDS and all showed evidence of reflux esophagitis.

The most common cardiovascular and respiratory symptoms reported by the patients were breathlessness on exertion (19, 61.3%), followed by cough and palpitation (11, 35.5%). Pulmonary function test with restrictive pattern was identified in 24 (77.4%) patients. The mean expected forced expiratory volume in one-second ($FEV_1$) was 66% ($\pm$ 19%) while the mean expected forced expiratory capacity was 58% ($\pm$ 17.9%). Almost a quarter of subjects had > 4% drop of oxygen level measured by finger pulse oximetry on 6MWT. The mean 6MWT distance was 328 ($\pm$ 75) metres. Of the 26 with HRCT or CXR performed previously, 22 (84.6%) had evidence of lung fibrosis. More than half of the study population had...
echocardiographic evidence of PAH (18, 58.1%) with five subjects having isolated PAH without lung disease. All patients had normal left ventricular ejection fraction and no history of congestive heart failure. Four (12.9%) subjects had right bundle branch block with ventricular ectopics.

SSc autoantibody profile

Table 3 shows the frequency, sensitivity and specificity of the antibodies in SSc patients and control subjects. Antinuclear antibodies were positive in 96.7% of SSc patients. Thirteen (41.9%) patients were positive for one antibody, nine (29%) were positive for two antibodies, whereas two (6.4%) were positive for three antibodies. The two most frequent autoantibodies identified were anti-Scl-70 (10, 32.3%) and anti-Ro-52 (10, 32.3%). ACA was present only in three (9.7%) patients. Seven (70%) patients with positive anti-Scl-70 antibody and seven (70%) with positive anti-Ro-52 antibody had evidence of lung fibrosis. The presence of anti-Scl-70 was significantly associated with restrictive lung disease pattern (P = 0.05). The presence of anti-PM-Scl-75 was associated with overlap syndrome (P = 0.032), and anti-CENP A antibody with vasculitic rash (P = 0.012). Among the healthy controls, three were positive for anti-PM-Scl-75, one was positive for Th/To and another for anti-Scl-70. Anti-Ro-52 antibody was significantly associated with control subjects with autoimmune diseases (P = 0.043). Anti-RP-11, anti-RP-155, anti-fibrillarin, anti-NOR-90 and anti-PDGFR had 100% specificity.

DISCUSSION

This study is one of the few existing published reports from Southeast Asia, where data on SSc are scarce.10–14 Malaysia is divided into western and eastern parts, separated by the South China Sea. The country has a multi-ethnic society comprising people of various racial backgrounds. According to the 2010 census report, Malaysian citizens consist of the ethnic groups Malay and indigenous (67.4%), Chinese (24.6%), Indian (7.3%) and other races (0.7%). Consistent with two previous local studies, one from West Malaysia and another from East Malaysia,10,11 there was a predominance of Chinese patients among those with SSc in the present study. Two other studies from the neighbouring country Singapore also reported the same finding.13,14 Genetic factors may play a role in this observation as human leucocyte antigen was closely linked to SSc susceptibility in China.15 However, the phenotypes of SSc appeared to be different in various regions of Asia. Studies in West Malaysia, India and Japan have reported more cases of lcSSc.10,16,17 In contrast, dcSS appears to be more commonly reported in East Malaysia, similar to the findings in Thailand, Singapore, Taiwan and China.11,12,14,18,19 A significant proportion of the subjects in our study demonstrated Raynaud’s phenomenon. Interestingly however, we observed a distinct pattern that features of CREST (calcinosis, Raynaud’s phenomenon, esophageal involvement, sclerodactyly and telangiectasia) were more commonly seen in cases of the diffuse cutaneous form. The lag time between onset of symptoms and

### Table 3

<table>
<thead>
<tr>
<th>Autoantibodies</th>
<th>SSc</th>
<th>Controls</th>
<th>Sensitivity%</th>
<th>Specificity%</th>
<th>Positive predictive value%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Scl-70</td>
<td>10 (32.2%)</td>
<td>2 (6.3%)</td>
<td>32.3</td>
<td>93.5</td>
<td>83</td>
</tr>
<tr>
<td>Anti-Ro-52</td>
<td>10 (32.2%)</td>
<td>6 (19.3%)</td>
<td>29.0</td>
<td>80.6</td>
<td>63</td>
</tr>
<tr>
<td>Anti-CENP-A (ACA)</td>
<td>2 (6.5%)</td>
<td>1 (3.2%)</td>
<td>6.5</td>
<td>96.8</td>
<td>67</td>
</tr>
<tr>
<td>Anti-CENP-B (ACA)</td>
<td>3 (9.7%)</td>
<td>1 (3.2%)</td>
<td>9.7</td>
<td>96.8</td>
<td>75</td>
</tr>
<tr>
<td>Anti-fibrillarin (U3RNP)</td>
<td>3 (9.7%)</td>
<td>0</td>
<td>9.7</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Anti-RP-11 (RNAP-III)</td>
<td>2 (6.5%)</td>
<td>0</td>
<td>6.5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Anti-RP-155 (RNAP-III)</td>
<td>2 (6.5%)</td>
<td>0</td>
<td>6.5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Anti-Ku</td>
<td>2 (6.5%)</td>
<td>2 (6.5%)</td>
<td>6.5</td>
<td>87.1</td>
<td>33</td>
</tr>
<tr>
<td>Anti-PM-Scl-75</td>
<td>2 (6.5%)</td>
<td>7 (22.5%)</td>
<td>6.5</td>
<td>83.9</td>
<td>29</td>
</tr>
<tr>
<td>Anti-PM-Scl-100</td>
<td>0</td>
<td>3 (9.7%)</td>
<td>NA</td>
<td>96.8</td>
<td>NA</td>
</tr>
<tr>
<td>Anti-Th/To</td>
<td>0</td>
<td>2 (6.5%)</td>
<td>NA</td>
<td>93.5</td>
<td>NA</td>
</tr>
<tr>
<td>Anti-PDGFR</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>100</td>
<td>NA</td>
</tr>
<tr>
<td>Anti-NOR-90</td>
<td>1 (3.2%)</td>
<td>0</td>
<td>3.2</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

ACA, anti-centromere antibody; RNAP-III, ribonucleic acid polymerase-III; PDGFR, platelet-derived growth factor receptor; NOR, nucleolus-organizing regions; NA, not available.
diagnosis varies in Malaysia, ranging from a mean of 14 months in our centre to 24.8 months in East Malaysia. The delay in diagnosis may be due to traditional medicine use, difficulty in healthcare access and under-recognition of this condition in primary care settings in East Malaysia.

Similar to other studies, symptoms of gastroesophageal reflux affected a significant proportion of our patients, especially in the dcSSc subgroup. The symptoms neither correlated with disease duration nor age of onset. One of our study limitations was that the EGDS results were retrospectively obtained and the total number was small. These have underestimated the prevalence of GERD in this study cohort. A separate study at our centre examining dysmotility and reflux using manometry and 24-h pH monitoring was recently completed. The provisional unpublished results suggested that a high proportion of patients have abnormal findings in the absence of symptoms. Interstitial lung disease also occurred frequently in our patients, especially among the diffuse cutaneous subset, a finding similar to a large European registry. Most patients with high PASP (> 30 mm Hg) on transthoracic echocardiography had underlying lung involvement. Compared to previous studies from Malaysia, a higher proportion of elevated PASP was observed in this study, which might be due to longer disease duration. It should be noted that abnormal echocardiographic findings were not further confirmed by right-heart catheterization because the cost of the procedure was beyond the study budget.

We evaluated the autoantibody profile of our subjects with Euroline immunoblot IgG. This assay was previously studied and showed a good agreement with conventional techniques for selected autoantibodies. Our study showed a high proportion of patients with more than one positive antibody, an observation that differs from Australian and German cohorts. Anti-Scl-70 was one of the two most commonly detected antibodies in this cohort. The antibody has been associated with organ manifestations, especially diffuse skin involvement and lung fibrosis. The majority of our patients with anti-Scl-70 had evidence suggestive of lung fibrosis. We did not find any significant association between this antibody and other organ manifestations, consistent with studies from Singapore and Canada. Anti-Ro-52, the other most commonly detected autoantibody in this study, is known to be associated with SSc with pulmonary involvement. The antibody is also present in Sjögren’s syndrome, rheumatoid arthritis and systemic lupus erythematosus.

The prevalence of the remaining antibodies (PM-Scl-75, PM-Scl-100, Th/To, PDGFR, RP-11, RP-155, Ku and NOR-90) was low, similar to the published data from other groups. Anti-PM-Scl antibodies, including anti-PM-Scl-75 and anti-PM-Scl-100, were highly specific for SSc (96.9%) in a large cohort of patients with SSc. The frequency of anti-Th/To and anti-PDGFR antibodies were between 2%–5% and 0–6%, respectively. Antibodies against PM-Scl-100, Th/To and PDGFR were not detected in any of our SSc patients, possibly due to the small sample size. Previous studies have suggested that anti-Th/To is related to lung fibrosis and scleroderma renal crisis, that is, a worse prognosis, and rarely found in other autoimmune diseases. Anti-PDGFR antibody plays a role as regulator in the fibrotic process. Anti-ribonucleic acid polymerase-III (anti-RNAP-III) (RP-11 and RP-155) confers more severe complications and defines those with diffuse disease and development of renal crisis. This antibody appears to be more prevalent among patients in Canada, Europe and Australia compared to Asia. Anti-Ku is generally seen in 2% of SSc patients and is associated with joint involvement and myositis. The clinical utility of anti-NOR-90 is limited.

Although systemic sclerosis is uncommon among Asians, we were able to characterize a small cohort of patients with SSc with notable similarities and differences between our centre and others in various geographic regions. Anti-Scl-70 remains the best characterized autoantibody and the most clinically useful. Anti-Ro-52 is a promising biomarker for pulmonary involvement of SSc. However, the roles of other more rare autoantibodies need further research. SSc is clearly a heterogeneous disease with marked geographical variation. To further understand the disease in Asia, a large epidemiological study should be conducted in the region.

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