Anti-Th/To-Positivity in a Cohort of Patients with Idiopathic Pulmonary Fibrosis

ARYEH FISCHER, FREDERICK J. PFALZGRAF, CAROL A. FEGHALI-BOSTWICK, TIMOTHY M. WRIGHT, DOUGLAS CURRAN-EVERETT, STERLING G. WEST, and KEVIN K. BROWN

ABSTRACT. **Objective.** To evaluate the presence and clinical relevance of anti-Th/To-positivity in patients with idiopathic pulmonary fibrosis (IPF).

**Methods.** Antinuclear antibody (ANA) testing was performed in 285 patients with a clinical diagnosis of IPF and surgical lung biopsy-proven usual interstitial pneumonia. Twenty-five subjects (8.8%) were found to have a positive ANA with a nucleolar-staining pattern and were followed for 10 years. Immunoprecipitation analysis indicated that 13 of the 25 subjects had autoantibodies against Th/To antigen.

**Results.** All subjects presented with worsening dyspnea. Pulmonary physiology and gas exchange did not differ between those with and those without a positive ANA, those with and without a nucleolar-staining ANA, and those with and without anti-Th/To antibody positivity. Retrospective review of the clinical record revealed that none of the 25 subjects with a nucleolar-staining ANA had the characteristic cutaneous features of systemic sclerosis (SSc). Four of the 13 Th/To-positive subjects had 3 of 5 criteria of limited cutaneous SSc (CREST variant), and 9 met proposed criteria for SSc sine scleroderma. None of the 12 Th/To-negative subjects had 3 or more criteria of limited cutaneous SSc (CREST variant), and only one met proposed criteria for SSc sine scleroderma. Of the 25 subjects with nucleolar-staining ANA, cumulative survival was similar between those who were Th/To-positive and those who were Th/To-negative (log-rank test, p = 0.73). Cumulative survival was similar between the 13 Th/To-positive subjects and all other 272 IPF subjects (log-rank test, p = 0.34).

**Conclusion.** Our findings indicate that a nucleolar-staining ANA is a common finding in patients with IPF, and that antibodies against Th/To are responsible for the majority of these. Given the high specificity of Th/To-positivity for SSc, our data suggest that these subjects may have SSc sine scleroderma, and that their prognosis is no different from those with IPF. (First Release June 15 2006; J Rheumatol 2006;33:1600–5)

Key Indexing Terms:

IDIOPATHIC PULMONARY FIBROSIS

ANTI-Th/To ANTIBODIES

The interstitial lung diseases (ILD) encompass a heterogeneous group of diffuse parenchymal lung diseases that can be divided into the primary or idiopathic disorders [e.g., idiopathic pulmonary fibrosis (IPF)] and a group of secondary disorders such as those associated with connective tissue diseases (CTD)\(^1\). ILD is a well known complication of a variety of rheumatologic diseases, with patients with systemic sclerosis (SSc) and rheumatoid arthritis being commonly affected. Indeed, ILD may be the first manifestation of a rheumatologic disease in general\(^2\), and SSc in particular\(^3\). It is estimated that about 15% of patients who present with ILD have, or will develop, a defined CTD\(^2\), with the initial clinical features of the lung disease being essentially indistinguishable from those of an idiopathic ILD\(^2,4\).

Antinuclear antibodies (ANA) have been found in > 90% of sera from patients with SSc\(^5\), with specific autoantibodies being associated with specific patterns of disease\(^6\). The anticientromere antibody (ACA) has been closely associated with limited cutaneous SSc (lcSSc), while anti-topoisomerase, or anti-Scl-70, the other commercially available SSc antibody, is
classically associated with diffuse cutaneous SSc (dcSSc). In addition to this, there is the less commonly occurring antinuclear antibody (ANoA) system, which comprises a mutually exclusive heterogeneous group of autoantibodies that produce nucleolar staining by immunofluorescence (IF)\(^\text{7,8}\). ANoA have been reported in 15–40% of patients with SSc\(^\text{6–10}\) but not in healthy controls\(^\text{7,8,11}\) or in healthy relatives of patients with SSc\(^\text{8,12}\). The most widely recognized of the ANoA system include anti-PM-Scl, anti-U3-RNP, anti-RNA polymerases I, II and III, and anti-Th/To\(^\text{8}\).

Anti-Th/To antibodies have been reported to occur in 4–13% of SSc patients\(^\text{13,14}\), occurring mostly in patients with lcSSc\(^\text{13,15}\). These patients have also been found to have significantly more radiographic evidence of ILD when compared with patients with ACA-positive lcSSc\(^\text{15}\).

We investigated the presence of nucleolar-staining ANA and anti-Th/To-positivity in a large cohort of patients with a clinico-pathologic diagnosis of IPF. We hypothesized that these patients would represent an unrecognized cohort of patients with SSc sine scleroderma.

**MATERIALS AND METHODS**

Subjects (Figure 1). Between 1985 and 1995, 285 patients referred for evaluation of an idiopathic ILD at National Jewish Medical and Research Center (NJMRC) in Denver, Colorado, had specific ANA testing performed as part of their initial evaluation. All subjects were enrolled in an institutional review board-approved protocol after informed consent was obtained. All 285 patients had surgical lung biopsy-proven usual interstitial pneumonia (UIP). On the basis of clinical features and surgical lung biopsy results, all subjects were diagnosed with IPF. Outcome was prospectively followed through January 2005. A retrospective chart review of the clinical evaluation was performed, and information on the following abstracted: history and physical examination, chest radiology, surgical lung biopsy results, and survival data.

Detection of antibodies. ANA were identified in the NJMRC laboratory by indirect IF testing in which HEp-2 cells (DiaSorin, Stillwater, MN, USA) were used as the substrate\(^\text{16,17}\). Serum dilutions of 1:40 were initially examined, and the pattern of IF was identified. Sera containing ANA were serially diluted to determine the endpoint titer. Since anti-Th/To antibody sera produce a bright nucleolar-staining pattern on the standard IF test\(^\text{14}\), we performed Th/To antibody immunoprecipitation on only those subjects with nucleolar-staining ANA. The identification of SSc-associated autoantibodies was done using immunoprecipitation assays as described\(^\text{16–18}\). Briefly, immunoglobulins in sera were allowed to adhere to protein A agarose beads (Invitrogen Life Technologies, Carlsbad, CA, USA). For the detection of autoantibodies against small nuclear ribonucleoproteins (snRNP), small cytoplasmic RNP, and Th/To, antigens were prepared from nonradio-labeled cells. Autoantigens were immunoprecipitated and separated by electrophoresis on denaturing acrylamide gels and detected by silver staining. Sera from healthy individuals were used as negative controls, and all autoantibodies were identified by comparison with standard sera.

Statistical analysis. We assessed differences between groups using t test (for continuous variables) and chi-square or Fisher’s exact test (for categorical variables). Survival curves were estimated using the Kaplan-Meier method and compared using the log-rank test; the date of lung biopsy was considered to be the reference time for survival. A critical significance level \(\alpha = 0.05\) or less was considered statistically significant. All data analyses were performed using SAS Version 9.1.3 (SAS Institute, Cary, NC, USA).

**RESULTS**

Detection of anti-Th/To antibodies (Figures 1, 2). During the defined study period, 285 subjects with IPF had an ANA as part of their laboratory evaluation. Ninety-seven subjects had a positive ANA of a titer > 1:40, and of these, 25 exhibited a nucleolar-staining pattern. Of these 25 serum samples, 13 tested positive for anti-Th/To.

Clinical features (Table 1). Of the nucleolar-staining ANA-positive subjects, all presented with the insidious onset of dyspnea and/or cough. None had skin changes diagnostic of SSc. The mean age at onset of ILD was similar between the Th/To-positive and Th/To-negative groups. As a group, the 13 Th/To-positive subjects had more features suggestive of underlying SSc: 4 exhibited digital edema, 2 had calcinosis, 5 had telangiectasia, and 9 had Raynaud’s phenomenon. Of the 3 subjects with documented nailfold capillaroscopy, one had findings consistent with SSc. Seven subjects had symptomatic gastroesophageal reflux disease (GERD), and 5 had pulmonary hypertension. In total, 4 subjects in the anti-Th/To-positive group had 3 of 5 criteria of the CREST (calcinosis, Raynaud’s, esophageal dysmotility, sclerodactyly, telangiectasias) variant of lcSSc and 9 met criteria for SSc sine scleroderma as proposed by Poormoghim, et al\(^\text{19}\).

Of the 12 subjects with nucleolar-staining ANA and anti-Th/To-negativity, none exhibited digital edema or calcinosis. One subject had telangiectasia, and one Raynaud’s phenomenon and associated nailfold capillaroscopy findings consistent with SSc. Five subjects had symptomatic GERD, and one had pulmonary hypertension. In total, no subject in the Th/To-negative group had 3 or more criteria of CREST and only one met proposed criteria for SSc sine scleroderma\(^\text{19}\).

Chest radiology and histopathology (Figures 3A, 3B). High resolution computed tomography (HRCT) data were indicative of various degrees of fibrosis (peripheral reticular and reticulonodular densities, linear fibrosis, and honeycombing), consistent with UIP pattern, in all subjects. Surgical lung biopsies were performed in all subjects and were consistent with UIP.

**Treatment.** All subjects received specific immunomodulatory treatment recommendations from their treating physician. Recommended therapy consisted of corticosteroids (CS) with or without cyclophosphamide (CYC) or azathioprine (AZA). Among the subjects in the Th/To-positive group, 3 subjects were treated with CS alone, 10 were treated with a combination of CS and a cytotoxic agent (5 with CYC, one AZA, and 4 CYC followed by AZA). Colchicine was used in 4 subjects. Among the group with nucleolar-staining ANA and Th/To-negativity, one was treated with CS alone, and 11 were treated with a combination of CS along with cytotoxic agents (6 with CYC, 2 with AZA, 3 with CYC followed by AZA). Colchicine was used in 5 of the subjects.

**Survival.** We found similar cumulative survival between the 13 Th/To-positive subjects [1963 days (95% CI 1420, 2339)] and all other 272 subjects with IPF [689 days (95% CI 550, 1000)] (log-rank test, \(p = 0.34\); Figure 4). We found similar
cumulative survival between those who were ANA-positive [735 days (95% CI 445, 1093)] and those who were ANA-negative [661 days (95% CI 494, 1119)] (log-rank test, p = 0.87); between those who were nucleolar-staining-ANA-positive [800 days (95% CI 445, 1110)] and those who were non-nucleolar-ANA-positive [2002 days (95% CI 467, 3001)] (log-rank test, p = 0.73).

**DISCUSSION**

Patients with ILD generally come to medical attention due to the onset of dyspnea and/or cough, with a diffusely abnormal chest radiograph prompting a more definitive evaluation. As ILD may be the first manifestation of a CTD in general and SSc in particular, the initial evaluation of a patient with ILD should include a history and examination as well as investigations that might suggest an underlying CTD, including pulmonary physiologic testing, thoracic HRCT imaging, and laboratory evaluation for ANA and rheumatoid factor (RF).

ANA are found in most patients with CTD, ranging from a prevalence of ~30% in rheumatoid arthritis to ~95% in patients with systemic lupus erythematosus (SLE) and SSc. However, positive ANA or RF are not specific for a CTD, as they are found in the general population and have been associated with various lung diseases including IPF.

Homma, et al prospectively followed 68 patients diagnosed with an idiopathic ILD over a period of 1–11 years. Thirteen patients (19%) subsequently developed systemic manifestations of a CTD. They reported that positive ANA and/or RF findings were not significantly different between the group of patients who developed CTD and those that did not. They did not look specifically for ANA. The authors concluded that patients clinically and/or histologically defined as having an idiopathic ILD could not be distinguished from CTD-associated ILD before the systemic manifestations of CTD appear.

ANA, however, are much more specific for SSc, and specific ANA have not been reported in healthy controls or in healthy relatives of SSc patients. However, the infrequency of these antibodies in SSc patient populations has limited their utility and they are not recommended in the diagnosis of SSc. The most widely recognized of the ANA system include anti-PM-Scl, anti-U3-RNP, anti-RNA polymerases I, II and III, and anti-Th/To.

Characterization of the Th/To antigen has been well docu-
mented. In 1982, Hardin, et al, reported that serum from a patient with SLE immunoprecipitated snRNP containing 7–2 RNA of a 32-P-labeled HeLa cell extract, distinct from other recognized RNP. The antibody responsible was designated “anti-Th.” Independently, Reddy, et al, in 1983, reported that a single antibody from a patient with SSc was found to immunoprecipitate RNP from Novikoff hepatoma cells that contained 2 RNA species, the 7–2 RNA described previously, and a novel cytoplasmic RNA, 8–2 RNA. This antibody was designated “anti-To.” It was later shown that Th and To antibodies were identical. In 1988, Gold, et al identified the 8–2 RNA as the RNA component of RNase P (the enzyme that processes all precursor transfer RNA transcripts to generate their mature 5’ termini). They later showed that the 7–2 RNA was identical to the RNA component of RNase MRP (a mitochondrial RNA-processing enzyme). Finally, in 2002, van Eenennaam, et al unequivocally defined the identity of the Th/To autoantigen and demonstrated that Th/To autoepitopes are found on several protein subunits of RNase MRP/RNase P.

The clinical significance of the anti-Th/To antibody is much less clear. Initially it was thought that these antibodies were relatively rare. In an effort to examine the array of ANA associated with SSc, Kipnis, et al studied 112 patients (38 with dcSSc, 31 with lcSSc, 28 with primary Raynaud’s phenomenon, and 15 with an overlap syndrome). Anti-Th/To antibodies were found in 6 patients with lcSSc, 4 with dcSSc, one with an overlap syndrome, and 2 with primary Raynaud’s phenomenon. Overall, anti-Th/To was found in 13% of the total number of patients; thus they concluded that this autoantibody occurs relatively commonly among SSc patients and its variants.

Okano and Medsger were the first to study the clinical, laboratory, and prognostic associations of anti-Th/To antibodies. Among 371 sera from SSc and SSc-overlap patients (152 of which had nucleolar-staining ANA), 15 (4%) were

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<th>Table 1. Demographic and clinical features of 25 subjects with ILD with nucleolar staining ANA who were Th/To-positive or Th/To-negative.</th>
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<td>Clinical Feature</td>
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<td>Mean age at ILD onset, yrs (range)</td>
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<td>F:M ratio</td>
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<td>Arthralgias</td>
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* In 1 of 3 examinations; ** in 1 of 1 examination. GERD: gastroesophageal reflux disease.

Figure 3. Photomicrographs of surgical lung biopsy from a Th/To-positive subject reveal findings typical of UIP: fibrosing interstitial pneumonia with heterogeneous interstitial fibrosis and focal honeycombing, fibroblast foci, alveolar pneumocyte hyperplasia, and minimal patchy lymphoplasmacytic infiltrate. A. H&E stain; original magnification 10×. B. H&E stain; original magnification 40×.
found to be positive for anti-Th/To (14 sera were from lcSSc patients and one was from a patient with dcSSc). Among 224 controls (consisting of SLE, polymyositis/dermatomyositis, SLE-myositis overlap, undifferentiated CTD, and primary Raynaud’s phenomenon), anti-Th/To was detected in only 3 (all having primary Raynaud’s phenomenon of < 2 years’ duration). They reported that the anti-Th/To subgroup had significantly greater frequencies of puffy fingers, small bowel involvement, and hypothyroidism, and a significantly lower frequency of arthralgias and/or arthritis. In addition, anti-Th/To-positive patients had a cumulative survival rate that was lower than that for patients who were anti-Th/To-negative.

More recently, Mitri, et al compared 87 anti-Th/To-positive patients to 306 ACA-positive patients with lcSSc. They reported that patients who were anti-Th/To-positive had more subtle skin changes, less severe vascular disease, and fewer had sicca findings or esophageal disease. Intrinsic pulmonary hypertension was found to be present in 28% of anti-Th/To-positive patients compared with 19% of ACA-positive patients. Th/To-positive patients were also more likely to have radiographic evidence of ILD (48% vs 13% of the ACA group) and SSc renal crisis. They also reported reduced survival in the Th/To group compared with that in the ACA group.

Our study differs from previous studies examining Th/To antibodies in that our study patients were taken from a large national referral center for ILD. After prospective evaluation, all of the patients were diagnosed with IPF. No patient had the characteristic cutaneous features of SSc. However, on retrospective evaluation, we found that many of the patients with nucleolar-staining ANA fit into the SSc spectrum of disease. Indeed, it appears that many of the patients with nucleolar-staining ANA met criteria for SSc sine scleroderma as proposed by Poormoghim, et al, and their ILD could be considered a forme fruste presentation of CTD. This was especially true among those who were Th/To-positive.

Interestingly, our study failed to show the expected better survival among those subjects identified to be within the SSc spectrum of disease. We did not find significant differences in survival among ANA-negative versus ANA-positive subjects, among nucleolar-staining ANA-positive versus non-nucleolar-ANA-positive subjects, nor among Th/To-positive versus Th/To-negative subjects. Indeed, our findings support the recent study by Kocheril, et al indicating that contrary to expectation, CTD-related ILD is not associated with a better prognosis than idiopathic ILD.

Our study has limitations. Using a retrospective medical record review in the absence of a prospectively defined and adhered-to evaluation scheme at the time of subject enrollment resulted in underreporting of potentially important clinical features in this patient population. And while the surgical lung biopsies in this cohort have been reviewed by more than one expert pulmonary pathologist to confirm the presence of UIP pattern, features of this pattern can overlap considerably with that of fibrosing nonspecific interstitial pneumonia, and given the ongoing evolution in the interpretation of these patterns, additional reviews might reclassify some of these biopsies as fibrosing nonspecific interstitial pneumonia.

Our findings indicate that a nucleolar-staining ANA is common in patients with usual interstitial pneumonia; moreover, the majority with this histologic pattern have Th/To autoantibodies. In addition, given the high specificity of Th/To-positivity for SSc and the retrospectively identified
clinical features in our patients, Th/To-positivity may identify a group of subjects with SSc sine scleroderma. Further prospective studies are necessary to determine whether Th/To antibody testing is useful in clinical phenotyping and to obtain useful data on the clinical course and prognosis of patients with interstitial lung diseases.

REFERENCES