Severe lung damage in anti-RNPC-3 antibody-positive patients: expanding the spectrum beyond systemic sclerosis

Sirs,

Anti-U11/U12 autoantibodies have been reported in patients with systemic sclerosis (SSc) (1). Some reports showed a strong association between anti-U11/U12 antibodies and interstitial lung disease (ILD), and a few reports noted that the antibodies were associated with cancer and/or moderate to severe gastrointestinal involvement in SSc patients (1). The U11/U12 RNP complex consists of several proteins with RNPC-3 considered the main target of the autoantibodies (1). Callejas-Moraga *et al*. showed the presence of anti-RNPC-3 antibodies to be associated with higher ILD frequency and either end-stage lung disease or death in a large cohort of Spanish and Italian SSc patients (2).

We investigated the prevalence of anti-RNPC-3 antibodies and the clinical characteristics associated with these antibodies in a Japanese cohort of 22 connective tissue disease (CTD)-ILD patients. The patients, diagnosed by the established criteria for each disease between November 2022 and September 2023, consisted of 18 SSc, two primary Sjögren's syndrome (SS), one dermatomyositis, and one rheumatoid arthritis patient. The study was approved by the ethics committees of Tosei General Hospital and Nagoya University Hospital and was conducted in accordance with the Declaration of Helsinki. All patients gave written informed consent.

This cohort comprised patients who were negative for routinely tested SSc- and

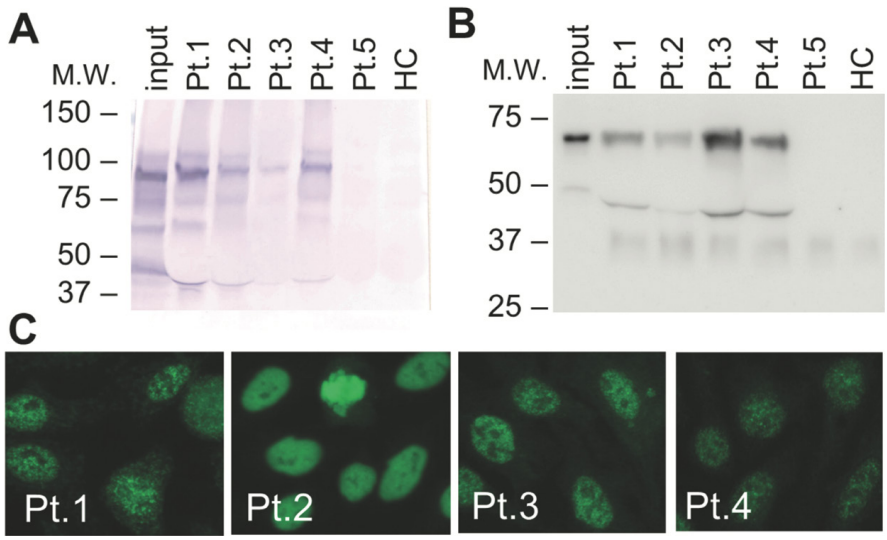

Fig. 1. Immunoprecipitation and indirect immunofluorescence staining with sera from anti-RNPC-3 antibody-positive patients.
A: Immunoprecipitation with recombinant RNPC-3 produced by *in vitro* transcription and translation.
B: Immunoprecipitates from K562 nuclear cell extracts with patient serum were probed with the anti-RNPC-3 monoclonal antibody (sc-514951, Santa Cruz, Dallas, USA). Input: a half-dose of the recombinant RNPC-3 protein produced with a cDNA clone (Product #FXC02057, Promega, Madison, WI, USA). Sera from patients 1-4 in Table I; serum from patient 5, an SSc patient with anti-RuvBL1/2 antibodies. HC: negative control serum from a healthy control.
C: Indirect immunofluorescence staining of HEp-2 cell slides (Fluoro HEPANA Test; MBL, Nagoya, Japan) with sera from anti-RNPC-3 antibody-positive patients shows the nuclear speckled pattern.

Table I. Detailed clinical features of the present patients with connective tissue disease and interstitial lung disease with the anti-RNPC-3 antibody.

	Patient 1	Patient 2	Patient 3	Patient 4
Sex	female	male	male	male
Age	58	53	61	51
CTD	SSc	Sjögren's syndrome	SSc	SSc
Diagnosis prior to referral	IIP	IIP	SSc	IPF
Initial SSc manifestation	ILD	ILD	skin, arthritis, RP, ILD	ILD
2013 ACR/EULAR SSc criteria score	9	5	16	9
mRSS (max.)	0/51	-	16/51	0/51
Arthritis	+	-	+	-
RP	+	+	+	+
SSc pattern on NVC	+	-	+	+
Digital ulcers or pitting scars	-	-	-	-
Calcinosis	-	-	-	-
GI involvement	+	+	+	+
Heart involvement	-	-	-	-
PAH	+	+	-	-
HRCT pattern	NSIP	NSIP	NSIP	NSIP
ATS pathological classification	mixture of NSIP and UIP	cellular fibrotic NSIP	mixture of NSIP and UIP	mixture of NSIP and UIP
FVC% predicted	60.0	85.2	95.3	67.3
DLCO% predicted	36.3	88.3	47.7	25.3
ILD staging	extensive	limited	extensive	extensive
KL-6, U/ml (max.)	2297	3443	3075	3779
Scleroderma renal crisis	-	-	-	-
Cancer	-	-	-	-
ILD treatment	immunosuppressive therapy	immunosuppressive therapy	antifibrotic therapy	combined immunosuppressive and antifibrotic therapy
Clinical course	initially stable, progressed to lung transplant	initial partial response, subsequent fibrosis progression	improved and stable long-term	initial partial response, progressed to lung transplant

The patient number corresponds to the number shown in Figure 1.
CTD: connective tissue disease; SSc: systemic sclerosis; IIP: idiopathic interstitial pneumonia; IPF: idiopathic pulmonary fibrosis; RP: Raynaud's phenomenon; ILD: interstitial lung disease; mRSS: modified Rodnan total skin thickness score; NVC: nailfold videocapillaroscopy; GI: gastrointestinal; PAH: pulmonary arterial hypertension; ATS: American Thoracic Society; NSIP: non-specific interstitial pneumonia; UIP: usual interstitial pneumonia; FVC: forced vital capacity; DLCO: diffusing capacity of the lung for carbon monoxide; KL-6: Krebs von den Lungen-6.

myositis-specific autoantibodies, including anti-centromere, anti-topoisomerase I, anti-RNA polymerase III, anti-MDA5, anti-TIF1-γ, anti-Mi-2, and anti-tRNA synthetases (anti-EJ, -Jo-1, -KS, -PL-7, and -PL-12 antibodies). Anti-OJ, anti-NXP-2, anti-SAE and anti-EIF2B antibodies were measured by our in-house ELISA, and all of the patients were also negative for all of these. We screened serum samples by using an ELISA that uses the recombinant RNPC-3 protein produced by an *in vitro* transcription/translation, as we reported (3). Highly reactive sera as detected by the ELISA were then tested by immunoprecipitation with the recombinant protein (Fig. 1A) and by Western blotting of the immunoprecipitated proteins using K562 nuclear extracts (3) (Fig. 1B).

We found 4/22 (18.2%) of the sera positive for the anti-RNPC-3 antibody: three from SSc patients (one diffuse cutaneous, two *sine* scleroderma) and one from an SS patient. Three serum samples from SSc patients exhibited a nuclear speckled staining pattern in indirect immunofluorescence studies, while an SS patient's serum showed a nuclear speckled and homogenous staining pattern (Fig. 1C). The clinical characteristics are summarised in Table I. All of the SSc patients showed nonspecific interstitial pneumonia (NSIP) pattern on HRCT, and surgical lung biopsy revealed a mixture of NSIP and usual interstitial pneumonia (4). All cases were classified as extensive ILD (5). The SS patient presented NSIP pattern on HRCT, with cryobiopsy revealing NSIP. Regarding treatment response, two SSc patients required lung transplantation and one improved with antifibrotic therapy; the SS patient experienced ILD progression after an initial treatment response.

Importantly, we identified anti-RNPC-3 antibodies in SSc *sine* scleroderma (ssSSc) cases and in primary SS without SSc. The previous study also found two anti-RNPC-3 antibody-positive patients with ssSSc (2). It was notable that our dermatology cohort had no anti-RNPC-3 antibody-positive patients among the 203 SSc patients (3). This result suggests that anti-RNPC-3 antibodies are frequently found in "lung-dominant" SSc patients. Anti-Ro52 antibodies also measured by our in-house ELISA (6), which may predict more severe lung damage in CTD-ILD (7), were not present in the anti-RNPC-3 antibodies-positive patients, but were found in four of the 18 patients without anti-RNPC-3 antibodies. None of our patients with anti-RNPC-3 antibodies were found to be complicated with cancer. All four patients exhibited 'mild' gastrointestinal involvement.

Despite the limitations of small sample size and selection bias, our findings warrant larger multicentre studies to confirm and establish the clinical significance of the anti-RNPC-3 antibody in CTD-ILD.

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