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Autoantibodies as biomarkers for interstitial lung disease in idiopathic inflammatory myositis and systemic sclerosis: The case of anti-eIF2B antibodies

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ABSTRACT

Objectives: Serum autoantibodies are pivotal for the early detection of systemic autoimmune rheumatic diseases such as Systemic Sclerosis (SSc) and Poly/Dermatomyositis (PM/DM), and in some cases are associated with organ complications such as interstitial lung disease (ILD). A paradigmatic example is provided by the autoantibody against the Eukaryotic Initiation Factor 2B (eIF2B) that has been recently detected in SSc.
Methods: Sera from 118 patients with SSc, 8 Poly/Dermatomyositis, 2 overlap SSc/Polymyositis, 4 undifferentiated connective tissue disease-UCTD and 3 healthy controls were tested first by indirect immunofluorescence for anti-nuclear antibodies-ANA pattern. Further, we employed prot
ein-radioimmunoprecipitation (IP) and IP- Western Blot for the detection and confirmation of anti-eIF2B antibodies. Serum findings were further correlated with the clinical features of patients.
Results: We identified 3 SSc cases (2.5%) positive for anti-eIF2B antibodies while this autoantibody was not detected in control sera. Using protein-IP all three patients manifested the 38kD protein which is the antigenic target of anti-eIF2B antibodies, and this was associated with a cytoplasmic pattern at indirect immunofluorescence. The presence of anti-eIF2B was associated with ILD and a diffuse SSc variant, in one case in association with anti-Scl70/topoI.
Conclusions: Our data confirm that a small subgroup (2.5%) of patients with SSc have detectable anti-eIF2B with cytoplasmic-positive staining at immunofluorescence and this reactivity is associated with ILD.

1. Introduction

Serum autoantibodies (autoAbs) are fundamental for the diagnosis and management of Systemic Sclerosis (SSc) and Poly/Dermatomyositis (PM/DM) [2,3], with rare ones often associated with specific clinical subsets [1] while more prevalent ones, such as anti-nuclear antibody (ANA), have a high sensitivity being found in nearly 100% of patients with connective tissue diseases. The prevalence of autoAbs in SSc and PM/DM is influenced by gender and ethnicity [4] [2,3,5] and therefore rare specificities need to be confirmed in different cohorts worldwide for clinical and prognostic associations [6,7]. In the last decades several new autoAbs have been discovered [6,8] with some becoming criteria for the classification of patients [9], such as anti-RNA polymerase III (RNApolIII) antibodies in SSc [10] and anti-Jo-1 in PM [11]. The presence of anti-Eukaryotic Initiation Factor 2B (eIF2B) in SSc was described in 2016 in 1–2% of patients with diffuse cutaneous disease and interstitial lung disease (ILD) and with negative ANA at indirect immunofluorescence (IIF) [12] and these observations were confirmed in a British study in 2018 [13].

We will herein discuss the main autoAbs associated with the risk of developing ILD in patients affected by SSc and PM/DM and report our original data on the prevalence and clinical significance of anti-eIF2B in SSc.

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Table 1
Main clinical and laboratory features of the 3 patients with SSc with positive anti-eIF2B antibodies.

	Patient #1	Patient #2	Patient #3
Sex	F	F	M
Age at present, years	65	75	88 (death from myocardial infarction)
Ethnic group	Caucasian	Caucasian	Caucasian
SSc variant	Diffuse	Limited	Diffuse
Interstitial lung disease	+	+	+
Gastrointestinal disease	+	–	–
Heart disease	+	–	+
Pulmonary hypertension	–	+	+
Digital ulcers	–	+	+
Skin calcinosis	–	–	–
Raynaud's phenomenon	+	+	+
Overlap	Myositis	–	–
Cancer	–	–	+ (Lung)
Pattern and titer by indirect immunofluorescence	Cytoplasmic 1:320	Cytoplasmic 1:320	Nuclear homogeneous + reticular cytoplasmic 1:1280

2. The case of anti-eIF2B in SSc

2.1. Subjects

We enrolled 118 consecutive patients affected by SSc, 2 PM/DM, 2 overlap SSc/PM, 4 undifferentiated connective tissue diseases (UCTD) and 3 healthy controls (HC) and sera collected in the years 2013–2017 were tested for anti-eIF2B antibodies. All patients with SSc fulfilled classification criteria [9] and the diffuse and limited variants were defined based on LeRoy criteria [14]; clinical and laboratory characteristics were derived from the medical records. ILD was determined by pulmonary high-resolution computed tomography (HRCT) in the presence of a ground-glass reticular pattern and honeycombing extent [15]. Serum samples were obtained from all patients and stored at −80 °C until used and at the same time clinical and laboratory data were collected. The study was approved by the Institutional Review Board of the hospital. This study met and was in compliance with the ethical standards of medicine, and informed consent was obtained from all patients in accordance with the Declaration of Helsinki and subsequent modifications.

2.2. Autoantibody detection

For autoAbs detection we used: (i) IIF using HEp-2 slides (INOVA, San

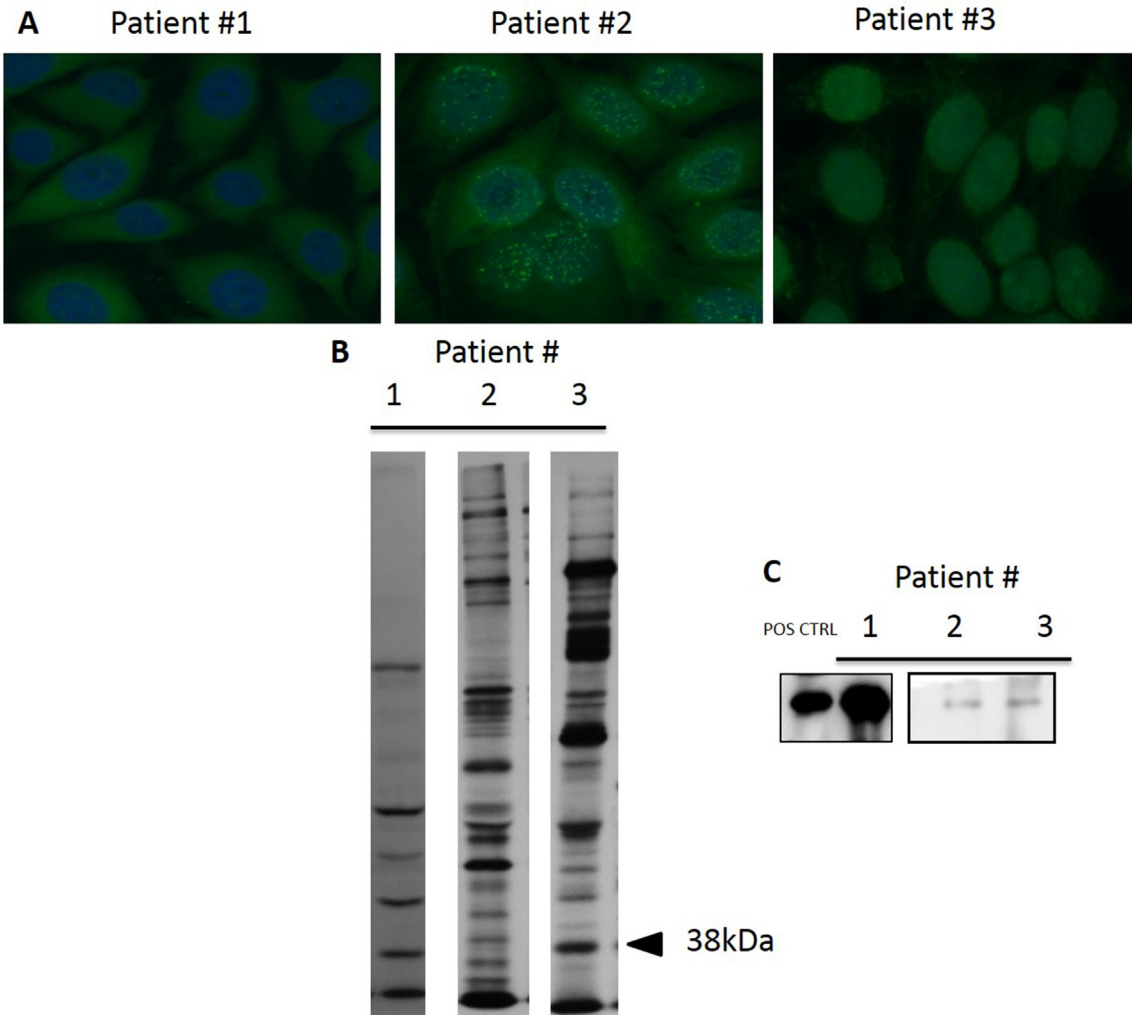

Fig. 1. Identification of anti-eIF2B in SSc shows (**Panel A**) IIF with a cytoplasmic staining in the 3 anti-eIF2B positive SSc cases, (**Panel B**) Protein-IP with the presence of the 38kD band which corresponds to the antigenic target of anti-eIF2B antibodies, with variable intensity, and (**Panel C**) IP-WB confirming the presence of eIF2B antigenic target, as represented by the positive control (POS CTRL) and the 3 anti-eIF2B positive SSc sera.

Diego, USA) with serum samples at 1:80 dilution incubated with goat anti-human IgG AlexaFluor488 AffiniPure F (ab')2 fragment (Jackson Immunoresearch Europe Ltd, Suffolk, UK) and DAPI for nuclear staining; (ii) protein-immunoprecipitation (IP) as previously described [8] to identify the 38 kDa band which is the target antigen for anti-eIF2B antibodies [12]; and (iii) IP- Western Blot (WB) on 26 SSc samples with 38-kD antigen band that has a possibility of eIF2B identified by protein-IP, using a specific anti-eIF2B antibody in 1:500 dilution (Novus Biological, Colorado, USA) for its confirmation.

2.3. Results

Through the use of the described methods, we identified 3/118 SSc sera (2.5%) positive for anti-eIF2B antibodies, and the main features of patients are described in Table 1 and shown in Fig. 1. Similar to reports from 2016 to 2018 [12,13], two of 3 anti-eIF2B-positive patients with SSc had a diffuse variant with coexisting ILD, while one had limited SSc with digital ulcers and Raynaud's phenomenon. One anti-eIF2B-positive case had an overlap syndrome of SSc and myositis, with no other serum autoAb associated, while patient #3 has a history of lung cancer and we also confirmed by IP positivity of anti-Scl70/topo I and anti-mitochondrial antibodies in this case. The IIF associated with anti-eIF2B antibodies was negative for ANA while all 3 anti-eIF2B-positive sera manifested a cytoplasmic staining at titers exceeding >1:320 (Fig. 1 **panel A**).

2.4. Discussion

We detected the presence of anti-eIF2B antibodies in 2.5% of SSc sera, supporting the rarity of this new autoAb described for the first time in 2016 [12]. Our results confirm that it is crucial to use specific laboratory techniques, namely protein-IP and IP-WB, to identify new and rare autoAbs such as the anti-eIF2B antibodies (Fig. 1 **panels B and C**). The most commonly detected autoAbs associated with SSc (directed against centromere, Scl70/topoI and RNApolIII) are found in as much as 50–70% of SSc cases, but the remaining 30–50% have anti-U3RNP/fibrillarin, anti-Th/To or unknown specificities that cannot be detected by routine methods and they require additional methods in research settings [5,10,16]. Through the combination of different testing methods, we could confirm that anti-eIF2B antibodies are present in different areas and ethnicity and not only in British SSc patients. This aspect is important because the prevalence of autoAbs in SSc patients can differ based on geographic areas, ethnic groups, and gender as demonstrated previously [2,17].

After confirming the 3 positive anti-eIF2B SSc cases, we analyzed their clinical and laboratory features as described in Table 1 and we confirmed the association of anti-eIF2B with diffuse SSc and ILD, and we did not detect anti-eIF2B antibodies in our control population. The overlap SSc-myositis was observed in one positive patient, and this is not surprising based on disease pathogenesis data [12], while another patient had a diagnosis of lung cancer and the association of anti-eIF2B antibodies with cancer may need further investigation to understand a possible link with cancer as for anti-RNApolIII [18]. IIF analysis showed a cytoplasmic staining in all 3 cases, at medium to high titer (Fig. 1 **panel A**), which is consistent with the cytoplasmic location of eIF2B, and also this aspect is concordant with previous reports [12,13]. One of our anti-eIF2B positive cases also had other autoAbs, namely anti-Scl70/topoI and anti-mitochondria antibodies as shown in Fig. 1 **panel B)** [5].

3. AutoAbs associated with ILD in SSc

It is well recognized that the presence of SSc-specific autoAbs confers a distinctive clinical profile and thus have a prognostic value at the moment of SSc diagnosis and clinical definition. In the setting of ILD, specific autoAbs have been reported in association with higher risk for this complication (Table 2), in particular anti-Scl70/topoI positivity ($p < 0.001$) [20–22] more than anti-centromere and anti-RNApolIII antibodies. Additional reports have confirmed that anti-Scl70/topoI autoAbs are strongly associated with diffuse SSc, ILD ($p < 0$ 0.001), pulmonary hypertension (PH) ($p = 0.019$) and ILD with PH ($p = 0.003$) while anti-CENPB are associated with limited SSc and inversely with ILD. Anti-Scl70/topoI have been associated with poor prognosis and higher mortality rate in severe SSc with ILD [23,24], thus their identification in the early onset of SSc is important to define specific follow-up examinations such as pulmonary function tests and HRCT. Univariable analysis revealed that male sex, presence of anti-Scl70/topoI and absence of anti-centromere were significant predictors of ILD [25]. In a Cox-regression analysis, a positive anti-centromere [hazard ratio (HR) 0.09 95% confidence interval (95% CI 0.01–0.73)] was confirmed to be a protective factor [25]. "Normal" FVC and CO diffusion in patients with SSc, especially those with positive anti-Scl70/topoI, should not obviate consideration of HRCT for ILD evaluation [26] (see Table 2).

Table 2
AutoAbs associated with ILD in SSc and additional clinical associations.

AutoAb	Prevalence (%)	SSc cutaneous variant	Clinical associations
Anti-Scl70/topoI	9.4–42	dSSc	ILD, renal crisis, digital ulcers
Anti-U3RNP	4–10	dSSc/lSSc	ILD, PH, renal crisis, myocardial fibrosis, gastrointestinal involvement, myositis
Anti-U11/U12 RNP	3	dSSc/lSSc	ILD, PH
Anti-Th/To	2–5	lSSc	ILD, PH, myositis
Anti-eIF2B	1	dSSc	ILD
Anti-CXCR3/4	–	dSSc	ILD
Anti-BICD2	–	lSSc	ILD and myositis
Anti-Ku	1.5–5	lSSc	ILD, myositis and arthritis
Anti-NOR90	4.8	lSSc	ILD, arthritis, sicca

Abbreviations: dSSc, diffuse SSc; ILD, interstitial lung disease; lSSc, limited SSc; PH, pulmonary hypertension.

Novel autoAbs have been detected in recent years and they have a prognostic role for specific clinical features (Table 2), similar to what observed for anti-eIF2B autoAbs in the British and Italian cohorts [12,13]. Anti-Th/To autoAbs are associated not only with ILD, but also with PH and inflammatory muscle disease [32,33]. The difference between limited SSc with anti-centromere versus anti-Th/To is that anti-Th/To-positive patients usually develop both ILD and PH. Moreover, pulmonary involvement is significantly more common in the anti-Th/To than in anti-centromere-positive patients (74% versus 51%, respectively) [32]. Anti-CXCR3/4 autoAbs and their corresponding receptors have been shown to be linked with the severity of SSc-ILD, and autoAb levels can discriminate patients with stable or decreasing lung function for risk stratification [27]. AutoAbs to BICD2 (bicaudal D2) also represent a new biomarker in SSc patients without other specific autoAbs, and data indicate that their major cross-reactive epitope is associated with anti-CENP-A but, unlike anti-CENP, single specificity anti-BICD2 antibodies associate with ILD and inflammatory myopathy [28]. Anti-Ku patients with elevated creatine-kinase at baseline may develop ILD and a diagnosis of SSc and not myositis [29], mainly, but not exclusively, in patients with diffuse SSc and with a speckled ANA pattern. Conversely, the presence of anti-centromere was considered protective against ILD as already mentioned [30]. Additional autoAbs associated with the risk of ILD in SSc include anti-RP11 and anti-NOR90, in particular in male patients [31], while patients with isolated SSc-PH are less likely to have anti-Scl70/topoI autoAbs and they are more prone to be ACA positive. Another serum reactivity that is more likely to be associated with SSc renal crisis and malignancy but less to ILD is the anti-RNApolIII [18,32].

Table 3
AutoAbs associated with ILD in PM/DM, and additional clinical associations.

AutoAb		Prevalence (%)	Disease association	Clinical associations
Aminoacyl tRNA synthetases				
	Jo-1	15–30	PM, DM	Anti-synthetase syndrome
	PL-7	<5	PM, DM	Anti-synthetase syndrome
	PL-12	<5	PM, DM, CADM	Anti-synthetase syndrome, ILD
	EJ	<5	PM, DM	Anti-synthetase syndrome
	OJ	<5	PM, DM	Anti-synthetase syndrome, ILD
	KS	<1	PM, DM	ILD
MDA5/CADM140		15–20	CADM/ADM	CADM, rapidly progressive ILD, severe skin manifestations

Abbreviations: CADM, clinically amyopathic dermatomyositis; DM, dermatomyositis; ILD, interstitial lung disease; PM, polymyositis.

4. AutoAbs associated with ILD in PM/DM

AutoAbs specific for idiopathic inflammatory myopathy (PM and DM) have been cumulatively coined "myositis-specific autoantibodies (MSA)" and are clinically useful biomarkers to help not only for the diagnosis but also for predicting and monitoring specific clinical manifestations such as ILD (Table 3) [5,33]. Anti-aminoacyl transfer RNA (tRNA) synthetases (anti-ARS) are a group of autoAbs that recognize the cytoplasmic amino acid-charging enzymes called "aminoacyl tRNA synthetases". So far, autoAbs to eight of them have been reported, including histidyl (Jo-1), threonyl (PL-7), alanyl (PL-12), glycyl (EJ), isoleucyl (OJ), asparaginyl (KS), phenylalanyl (ZO), and tyrosyl (YRS/HA) tRNA synthetases [3]. A report published in 2014 by Nakashima et al. reported that 10% of patients with idiopathic ILD had anti-aminoacyl transfer RNA (tRNA) synthetases (anti-ARS) [34], which is consistent with the fact that anti-ARS have a strong association with ILD and ILD may precede myositis in some cases. Nevertheless, some patients appear to have idiopathic ILD for years and may not develop myositis.

Reports on anti-PL-12 and anti-KS suggested that they are common in ILD without myositis [35–37], as reported also by Kalluri and Colleagues who analyzed the clinical features of 31 anti-PL-12-positive patients and reported that this autoAb is strongly associated with ILD (3/31 cases were idiopathic ILD) but less with myositis and arthritis [38], consistent with an earlier study [35,37]. Marie et al. analyzed 75 anti-Jo-1-positive patients and 20 anti-PL-7 (n = 15)/PL-12-positive (n = 5) patients and reported that the latter had milder muscle involvement, less recurrence of muscle disease and anti-PL-7/PL-12 was associated with early and severe ILD and gastrointestinal manifestations [39,40]. Hamaguchi et al. compared the clinical features of patients with different anti-ARS and reported that CADM or ILD were more associated with anti-PL-12, and ILD with anti-KS and anti-OJ antibodies [41]. In summary, patients with antibodies to non-Jo-1 ARS are associated with earlier and more severe ILD compared with anti-Jo-1 (+) patients, and they are more likely to have ILD without typical myositis [3].

Besides ARS, another autoAb associated with ILD is anti-MDA5/CADM140 which has been reported in association with rapidly progressive ILD resistant to treatment and with poor prognosis (Table 3) [42–44]. In a study published by Hall et al. [45], 6.9% (11/160) of DM had anti-MDA5/CADM140 but 6/11 had overt clinical myopathy and 8/11 had ILD. This cohort of anti-MDA5/CADM140 patients was similar to anti-synthetase syndrome and they were not associated with RPILD, in contrast to Asian studies. Another study from Spain reported 12% (14/117, 8 were CADM) prevalence of anti-MDA5/CADM140 in 117 DM patients [46].

Eight of 14 anti-MDA5/CADM140-positive patients had RPILD, similar to Asian cohorts [47] but different from US cohorts [45].

5. Conclusions

Research techniques are fundamental for the identification of new and rare autoAbs in SSc, as demonstrated by the recent description of anti-eIF2B antibodies. The data confirm the association of this autoAb with SSc, and it helps understanding the future possible disease development for a better clinical and therapeutic management, as reported previously for other SSc autoAbs [19]. Anti-eIF2B is the autoAb specificity that should be suspected when SSc patients are negative for anti-centromere, -Scl70/topoI, -RNApolIII antibodies but have positive cytoplasmic staining with negative ANA at IIF. Increasing the number of anti-eIF2B positive patients and understanding their significance in the SSc disease is of great importance to better manage patients and to allow the use of this specificity in routine autoimmunity laboratories worldwide.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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