Clinical spectrum time course in non-Asian patients positive for anti-MDA5 antibodies

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Abstract

Objective
To define the clinical spectrum time-course and prognosis of non-Asian patients positive for anti-MDA5 antibodies.

Methods
We conducted a multicentre, international, retrospective cohort study.

Results
149 anti-MDA5 positive patients (median onset age 53 years, median disease duration 18 months), mainly females (100, 67%), were included. Dermatomyositis (64, 43%) and amyopathic dermatomyositis (47, 31%), were the main diagnosis; 15 patients (10%) were classified as interstitial pneumonia with autoimmune features (IPAF) and 7 (5%) as rheumatoid arthritis. The main clinical findings observed were myositis (84, 56%), interstitial lung disease (ILD) (108, 78%), skin lesions (111, 74%), and arthritis (76, 51%). The onset of these manifestations was not concomitant in 74 cases (50%). Of note, 32 (21.5%) patients were admitted to the intensive care unit for rapidly progressive-ILD, which occurred in median 2 months from lung involvement detection, in the majority of cases (28, 19%) despite previous immunosuppressive treatment. One-third of patients (47, 32% each) was ANA and anti-ENA antibodies negative and a similar percentage was anti-Ro52 kDa antibodies positive. Non-specific interstitial pneumonia (65, 60%), organising pneumonia (23, 21%), and usual interstitial pneumonia-like pattern (14, 13%) were the main ILD patterns observed. Twenty-six patients died (17%), 19 (13%) had a rapidly progressive-ILD.

Conclusion
The clinical spectrum of the anti-MDA5 antibodies-related disease is heterogeneous. Rapidly-progressive ILD deeply impacts the prognosis also in non-Asian patients, occurring early during the disease course. Anti-MDA5 antibody positivity should be considered even when baseline autoimmune screening is negative, anti-Ro52 kDa antibodies are positive, and radiology findings show a NSIP pattern.

Key words
melanoma differentiation-associated protein 5 antibody, rapidly progressive interstitial lung diseases, idiopathic inflammatory myopathies

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Introduction

The availability of commercial kits for the determination of myositis-specific antibodies (MSA) has progressively changed the rheumatologic approach to idiopathic inflammatory myopathies (IIMs), in terms of classification, follow-up, and treatment (1-6). Furthermore, MSA positivity is frequently associated with the occurrence of interstitial lung disease (ILD), as evidenced in the interstitial pneumonia with autoimmune features (IPAF) classification criteria (7). Among the different MSA, anti-MDA5 antibodies are at high risk for the occurrence of a disease-related acute respiratory failure, defined Rapidly progressive-ILD (RP-ILD), a dreadful form of lung involvement mainly described in Asian patients (8-15). Only a few studies focused on anti-MDA5 antibodies in non-Asian cohorts (8, 14, 16-19), and a better definition of the clinical characteristics and evolution of these patients in non-Asian settings is necessary. This retrospective study aims to describe the clinical features and outcome of anti-MDA5 antibodies in a large cohort of non-Asian patients from European and American centres referring to the AENEAS (American and European NEtwork of Antisynthetase Syndrome) collaborative group.

Methods

Patients

After approval of the Ethical Committee of the IRCCS Policlinico San Matteo Foundation of Pavia, Italy, data were retrospectively collected from January to November 2019. Inclusion criteria were anti-MDA5 positivity along with at least one feature among arthritis, myositis, ILD, and typical skin lesions. Disease onset was considered from the observation of the first pulmonary, muscle, joint, or skin symptom/sign. Features' onsets were defined concomitant when they occurred less than 3 months apart. Rapidly progressive-ILD (RP-ILD) was defined as an ILD requiring intensive care unit (ICU) admission due to disease-related acute respiratory failure, after the exclusion of other possible causes (pulmonary infections, heart failure, embolism). According to lung involvement, patients were categorised into 3 different groups: patients without ILD ("no ILD"), with ILD but without RP-ILD ("ILD"), and with RP-ILD ("RP-ILD").

Definition of manifestations

ILD was defined by evidence of alveolitis/fibrosis on chest high-resolution computed tomography (HRCT). In the case of pulmonary function tests (PFTs) availability, the occurrence of a restrictive pattern and/or the impairment of DLCO was considered as additional markers of ILD.

Muscle involvement was identified by muscle enzyme elevation (creatinine phosphokinase and/or aldolase increase>50% with respect to the upper limit of normal) and typical electromyography and/or muscle biopsy and/or muscle magnetic resonance alterations. Myositis onset was defined as classic (proximal muscle weakness) or hypomyopathic (instrumental/laboratory evidence of muscle impairment without muscle weakness).

Arthritis was intended as joint swelling/tenderness, excluding non-inflammatory joint involvement due to osteoarthritis, fibromyalgia, ligament/tendon disease.

Skin lesions included the occurrence of dermatomyositis typical lesions (Gottron papules/sign, mechanic's hands, Hiker's feet, heliotrope rash, shawl, and V sign, skin ulcers). Fever was regarded as a body temperature ≥38°C for more than 10 days without other explanation than disease activity. Raynaud's phenomenon (RP) was defined as transient finger discolouration after cold exposure, confirmed by a clinician.

Laboratory tests

Anti-MDA5 antibodies were considered positive only if the evaluation was performed in the leading reference/tertiary centre that included the patient. The Euroline Autoimmune Inflammatory Myopathies 16 Ag kit (Euroimmun, Luebeck, Germany) was the only kit used for anti-MDA5 antibodies determination in different sites. Anti-MDA5 antibodies were interpreted only as positive or negative according to the cut-offs established by the manufacturer, to reduce potential local bias in autoantibodies evaluation. Antinuclear antibodies (ANA) were evaluated by
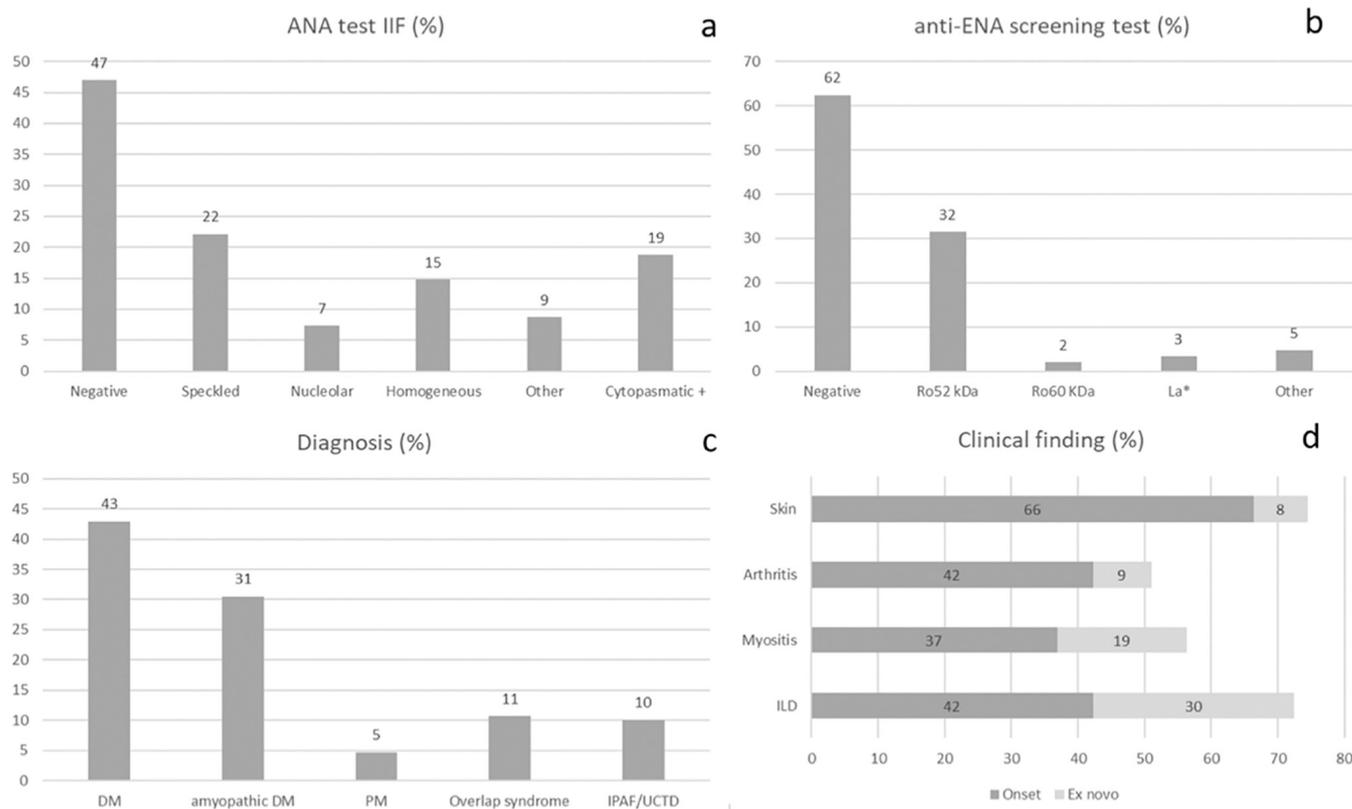
Fig. 1. Prevalence of ANA in the 149 enclosed patients, including the assessment of cytoplasmic positivity (a), anti-ENA antibodies (b), diagnosis (c), and clinical findings (d).

DM: dermatomyositis; PM: polymyositis; IPAF: interstitial pneumonia with autoimmune features; UCTD: undifferentiated connective tissue disease; ILD: interstitial lung disease; ANA: antinuclear antibodies; anti-ENA: anti-extractable nuclear antigens.

indirect immunofluorescence (IIF) and considered positive only in the case of titre ≥1/160. Anti-extractable nuclear antigens (ENA) antibodies assessment was performed with the locally available routine screening tests.

Statistical analysis
The patients' characteristics at disease onset and last follow-up were reported using median and interquartile range (IQR) for the quantitative variables and absolute/relative frequency values for the qualitative ones. Overall comparison among groups was performed by the one-way ANOVA or by non-parametric Kruskal-Wallis test for quantitative variables, and by χ^2 or Fisher's exact test for categorical variables; significant differences between groups were evaluated in a head-to-head comparison. Survival was estimated using the Kaplan-Meier method and comparisons between groups were made by the log-rank test. Analyses were performed using the STATA software package (2018, release 15.1; StataCorp, College Station, TX).

Results
General characteristics
We included 149 patients (100 females, 67%, 49 males, 33%). Median age at disease onset was 53 years (Interquartile range, IQR, 41–62), median diagnostic delay 4 months (IQR 7–51), and median disease duration 18 months (IQR 7–51). Patients were diagnosed with dermatomyositis (n=64, 43%), amyopathic dermatomyositis (n=47, 31%), overlap myositis (n=16, 11%), polymyositis (n=7, 5%) and interstitial pneumonia with autoimmune features (IPAF) (n=15, 10%) (Fig. 1C). All patients diagnosed with polymyositis and dermatomyositis were classifiable as probable or definite IIMS according to the 2017 ACR/EULAR classification criteria (20). IPAF patients did not satisfy any other existing classification criteria for mimicking conditions. Thirty-seven patients (25%) satisfied the 2010 ACR/EULAR classification criteria for rheumatoid arthritis (RA) (21), but only 7 were considered as in overlap with RA (5%).

Clinical findings
- Interstitial lung disease
ILD was identified in 108 patients (72%), RP-ILD in 32 (21.5%). Chest high resolution computed tomography (HRCT) (Fig. 2) mainly showed findings of non-specific interstitial pneumonia (NSIP), (65 patients, 60% of cases with ILD; 19 with RP-ILD, 59% of "RP-ILD" group), organising pneumonia (OP) (23 patients, 21% of cases with ILD; 8 with RP-ILD, 25% of "RP-ILD" group), and usual interstitial pneumonia (UIP)-like pattern (14 patients, 13% of cases with ILD; 4 with RP-ILD, 13% of "RP-ILD" group). Of note, 10 patients (9% of cases with ILD) had both NSIP and OP findings, of whom 5 developed RP-ILD (16% of "RP-ILD" group). Only 2 patients with RP-ILD (6% of "RP-ILD" group) had evidence of diffuse alveolar damage (DAD) at lung HRCT. Median forced vital capacity (FVC) at ILD onset was 80% of theoretical values (IQR 67–92%) and DLCO 56% (IQR 45–69%). All ILD patients with available PFTs had at least DLCO impairment.

Fig. 2. Prevalence of different HRCT pattern of interstitial lung involvement (n=108 patients) in both ILD and RP-ILD group.

NSIP: non-specific interstitial pneumonia; OP: organising pneumonia; UIP: usual interstitial pneumonia; DAD: diffuse alveolar damage; HRCT: high resolution computed tomography; ILD: interstitial lung disease.

Percentages are expressed with respect to the overall number of patients with interstitial lung involvement (in bold the overall prevalence of patterns).
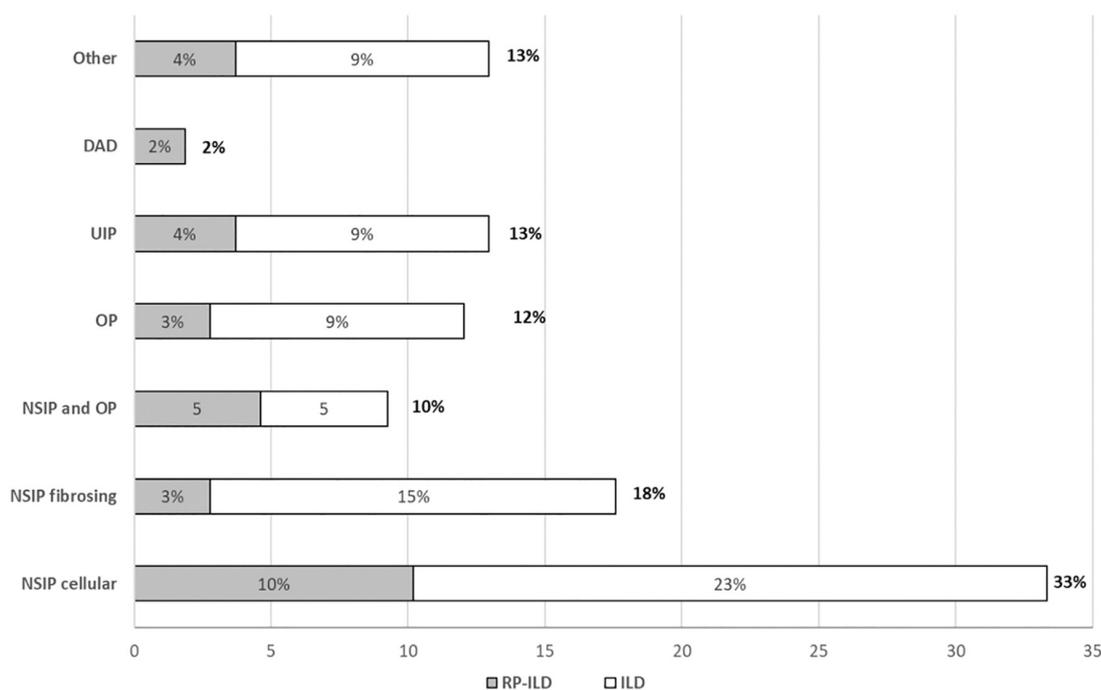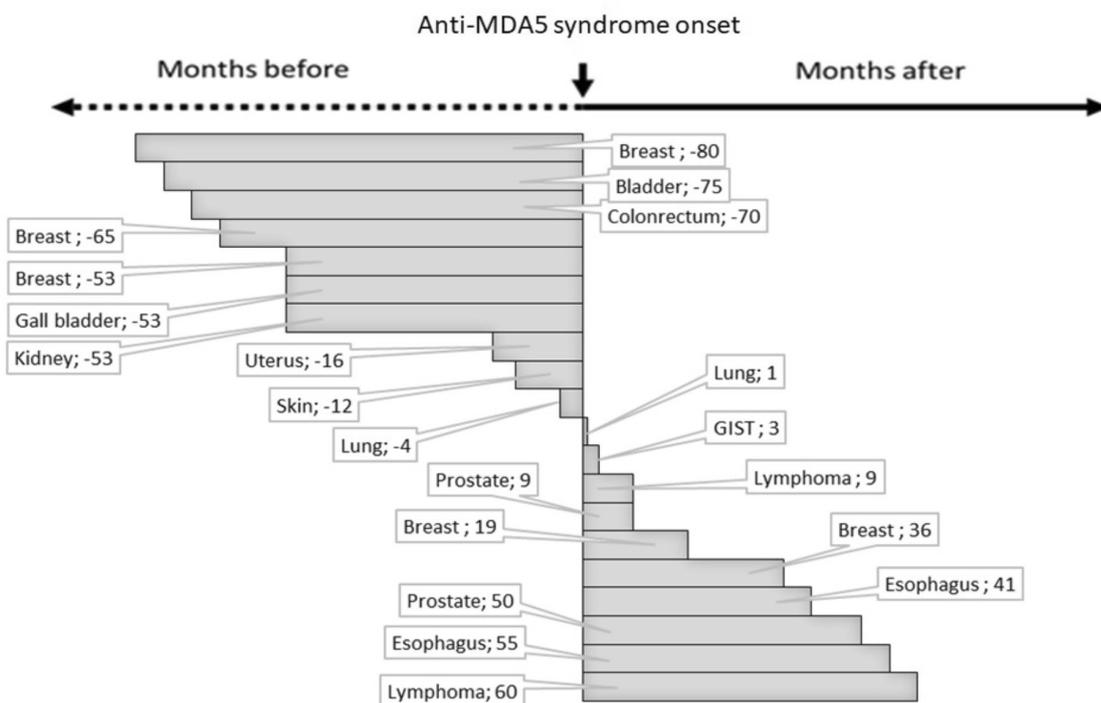

Fig. 3. Time-relationship between cancer and anti-MDA5 syndrome diagnosis.

In the callout we reported cancer site or type and the timing of appearance, in months, of the cancer with respect to anti-MDA5 diagnosis.

- Non-lung findings

Muscle involvement was common (n=84, 56%) and mainly symptomatic (n=73, 49%), as well as joint involvement (n=76, 51%). Joint involvement was generally polyarticular and symmetrical (n=59, 78% of patients with arthritis). Most patients had skin involvement (n=113, 74%). Skin ulcers were reported in 22 cases (15%). RP (n=45,

30%) and fever (n=43, 29%) were rarely evidenced, sometimes concomitantly (n=11, 7%). Twenty patients (13%) had a history of cancer (Fig. 3), in all but two cases of solid type, mainly breast cancer (n=5, 25%). Only 9 neoplasms (45%) occurred in the 36 months before or after the onset of the anti-MDA5 syndrome, and they could be considered disease-related cancer. Furthermore,

only 6 neoplasms (30%) occurred in the 12 months before or after the onset of the anti-MDA5 syndrome, a timeframe that strongly supports the effective relationship between disease and cancer.

- Clinical spectrum time course

Arthritis, myositis, ILD, and skin involvement had a delayed appearance in 74 patients (50%), and the main *ex-novo*

finding occurring during the follow-up was ILD (n=45, 39%), followed by myositis (n=29, 19%), arthritis (n=13, 9%) and skin lesions (n=12, 8%) (Fig. 1D). The main clusters of manifestations at disease onset were isolated skin findings (n=21, 14%), and skin findings + ILD (n=19, 13%). At last follow-up patients presented mainly skin findings + arthritis + myositis + ILD (n=28, 19%), skin findings + ILD (n=21, 14%), and skin findings + arthritis + ILD (n=19, 13%). In Supplementary Figure S1 we reported the cluster of clinical findings at disease onset and at last follow-up.

- Autoimmune profile
ANA test was positive in 79 patients (53%), mainly with a speckled pattern (n=33, 22%), ANA cytoplasmic positivity was observed in 27 cases (19%) (Fig. 1A). Anti-ENA antibodies were detected in 56 patients (38%), mainly with anti-Ro 52kDA specificity (n=47, 32%) (Fig. 1B). Some patients were rheumatoid factor (n=10, 7%) and anti-cyclic citrullinated peptide antibodies (n=6, 4%) positive. Forty-seven patients (32%) were negative for both ANA (including the cytoplasmic fluorescence) and anti-ENA screening, whereas 42 (28%) were also negative for rheumatoid factor and anti-cyclic citrullinated peptide antibodies.

- Subgroup analysis
Forty-one patients (27.5%) were included in the "non-ILD" group, 76 (51%) in the "ILD" group, and 32 (21.5%) in the "RP-ILD" group (Table I).
In the "RP-ILD" group the median time-lag to ICU admission was 6 months (IQR 3–10.5) from disease onset and 2 months (IQR 0–7.5 months) from ILD detection. In the first 12 months from ILD onset, 27 out of 108 patients with ILD (25%) developed RP-ILD. Forty-nine ILD patients had more than 12 months of disease duration and were exposed to the risk of RP-ILD occurrence. Five of these patients (10%) developed RP-ILD. RP-ILD was statistically more common in the first 2 months from ILD onset (*p*=0.032) than in the subsequent follow-up period. Of note, only one patient was admitted to ICU more than 18 months after ILD onset (at month 27).

Groups' comparisons substantially showed that "non-ILD" patients were more commonly females (*p*<0.01 *vs.* other groups), and younger (*p*=0.006 *vs.* "RP-ILD"). "RP-ILD" patients had a shorter follow-up (*p*<0.01 *vs.* other groups), presented more frequently anti-Ro52 kDA positivity (*p*=0.006 *vs.* "non-ILD"), lymphopenia (*p*=0.015 *vs.* "ILD"), and fever (*p*<0.001 *vs.* other groups). Only a few patients had a history of smoking exposure (10, 24%, "non-ILD"; 18, 24%, "ILD"; 6, 19%, "RP-ILD"; *p*=0.102). Ferritinemia dosage (median 1324 ng/ml, IQR 705-2097) was fully available only in "RP-ILD" patients, whereas other groups had a relevant number of missing values, that did not allow the comparison. HRCT patterns of lung involvement at presentation, reported in Figure 2, were not statistically different between ILD and RP-ILD patients (*p*=0.213). The median FVC% at diagnosis was 79% (IQR 67-90) in the "ILD" group and 73% (IQR 64-87) in the "RP-ILD" (*p*=0.855, unpaired sample t-test). The median DLCO% at diagnosis was 57% (IQR 44-70) in the "ILD" group and 58% (IQR 50–67) in the "RP-ILD" (*p*=0.753, unpaired sample t-test). PFTs and DLCO were not available in 7 RP-ILD patients because of a very acute onset of the condition.

- Evolution and outcome
Twenty-six patients died during the follow-up (17%), 19 of them (73%) were in the "RP-ILD" group, 2 (8%) in the "non-ILD" group, and 5 (19%) in the "ILD" group (Fig. 4). "RP-ILD" was the direct cause of death in 12 patients (46% of all deaths), other 5 patients (19%) died for infectious complications of immunosuppressive treatment (in particular one patient 2 months after lung transplantation), one patient (4%) died 18 months after ICU discharge due to chronic progression of ILD and another one (4%) died for suffocation, due to deglutition muscle involvement. Of note, among the 13 patients (9% of total) that had an isolated ILD at disease onset, 4 (31% of isolated ILD) developed RP-ILD, and 3 of them died. One (4%) "RP-ILD" patient who died due to respiratory failure was diagnosed with

colorectal cancer at the autopsy. One (4%) "ILD" patient died 23 months after disease onset due to respiratory failure in the setting of slowly progressive respiratory involvement. Two patients (8%) died for cancer progression (one "non-ILD" and one "ILD"). In 4 cases (16%) the causes of death were not defined (3 in the "ILD" and 1 in the "non-ILD" group), but RP-ILD was excluded in all these cases. Only 3 out of 149 patients (2%) were lost to follow-up, after 8, 10, and 51 months respectively. The Kaplan-Meier survival curve is reported in Figure 5. The log-rank test confirmed that survival was significantly reduced in the "RP-ILD" group (*p*<0.001 at month 12, 18, 36, and overall). Treatments performed in the "RP-ILD" cohort, during ICU hospitalisation and in the 6 months before the admission, are reported in Table II. In the 6 months before ICU admission, 28 RP-ILD patients (87%) were treated with immunosuppressants. Most of them were treated with steroids (10, 31%), even in pulses (4, 13%), whereas cyclosporine, mycophenolate mofetil, cyclophosphamide (IV), and rituximab (IV) were used in 6 cases each (19%). Six patients (19%) were also treated with high-dose intravenous immunoglobulins, whereas 4 patients had an onset directly in ICU, without previous immunosuppressive treatment. In ICU, patients were treated with steroid pulses (18, 56%), cyclophosphamide IV (14, 44%), rituximab (10, 31%), cyclosporine (8, 25%) and mycophenolate mofetil (6, 19%), in some cases even in sequence. Intravenous high dose immunoglobulins were used in 8 cases (25%), whereas extra corporeal membrane oxygenation was adopted in 6 cases (19%). No patients were treated with plasmapheresis, event those on ECMO. One patient underwent lung transplantation but died two months later because of lethal infection. In one patient the disease course was so aggressive that it was not possible to start any immunosuppressive treatment. The drugs with the best performance, based on the rate of patients alive after hospital dismission and without subsequent chronic progression of the disease, were mycophenolate mofetil (5 responders, 1 not responder, 83%), and

Table I. Main patients' characteristics according to different sub setting (patients without ILD; patients with ILD but without RP-ILD; patients with RP-ILD). If not otherwise specified, percentages are referring to correspondent column population.

	No ILD		ILD		RP-ILD		P (significance < 0.05)
Number of patients (% of the cohort)	41	(27.5)	76	(51)	32	(21.5)	-
Females (%)	35	(85)	49	(64)	16	(50)	0.005*
	reference		p=0.017*^		p=0.001*^		
Median age at disesse onset (IQR)	44	(32 - 56)	53	(42 - 64)	58	(49 - 68)	0.006©
	reference		p=0.052		p=0.006		
Median diagnostic delay (IQR)	5	(2 - 12)	3.5	(2 - 9)	3	(2 - 8)	0.705£
Median follow-up (IQR)	24	(8 - 98)	21	(9 - 56)	8	(4 - 21)	0.001£
	p=0.002		p<0.001		reference		
ANA test negative (%)	22	(54)	34	(45)	14	(44)	
ANA test pattern speckled (%)	10	(24)	18	(24)	5	(16)	
ANA test pattern homogeneous (%)	6	(5)	8	(10)	8	(25)	0.170§
ANA test pattern nucleolar (%)	3	(7)	7	(9)	1	(3)	
ANA test other patters (%)	0	(0)	9	(12)	4	(12)	
ANA cytoplasmic positivity (%)	5	(12,2)	16	(21,1)	7	(21,9)	0.444
Anti-Ro52 kDa positivity	7	(17,1)	25	(32,9)	15	(46,9)	0.023*
	p=0.006^		p=0.170*^		reference		
Lymphopenia	20	(49)	26	(34)	19	(59)	0.041*
	p=0.124*^		p=0.015*^		reference		
Muscle involvement (%)	31	(76)	34	(45)	19	(59)	0.005*
	p=0.001*^		reference		p=0.165*^		
Symptomatic (% of patients with muscle involvement)	26	(84)	28	(82)	19	(100)	0.138*
Asymptomatic (% of patients with muscle involvement)	5	(16)	6	(18)	0	(0)	
Arthritis	21	(51)	42	(39)	13	(41)	0.381*
ACR/EULAR 2010 RA classification criteria, number (% of arthritis patients)	14	(67)	16	(38)	7	(54)	0.093*
Skin involvement (%)	28	(68)	58	(76)	25	(78)	0.553*
Raynaud's phenomenon	12	(29)	25	(33)	8	(25)	0.416*
Fever (%)	9	(22)	15	(20)	19	(59)	<0.001*
	p<0.001*^		p<0.001*^		Reference		
Dermatomyositis (%)	21	(51)	27	(35)	16	(50)	
Amyopathic dermatomyositis (%)§	7	(17)	31	(41)	9	(28)	
Polymyositis (%)	3	(7)	3	(4)	1	(3)	0.099§
Overlap myositis (%)§	8	(20)	6	(8)	2	(6)	
IPAF/UCTD (%)	2	(5)	9	(12)	4	(13)	

ILD: interstitial lung disease; RP-ILD: rapidly progressive interstitial lung disease; ANA: antinuclear antibodies; RA: rheumatoid arthritis; IPAF: interstitial pneumonia with autoimmune features; UCTD: undifferentiated connective tissue disease.
Statistical analysis: *χ^2; §Fisher's exact test; © one-way Anova with Bonferroni *post-hoc* test; £Kruskal-Wallis test. ^significant *p*-value <0.025.

rituximab (5 responders, 5 not responders, 50%). The drugs with the worst performance were cyclophosphamide (5 responders, 9 not responders, 36%), cyclosporine and high dose intravenous immunoglobulins (for both 1 responder, 7 not responders, 12.5%).
Regarding the treatment of other groups, non-ILD patients (n=41) were treated with corticosteroids in 39 cases (95%), that were administered as IV bolus (methylprednisolone 1 g/day for 3 consecutive days) in 5 cases (12%). Hydroxychloroquine was prescribed in 20 cases (50%) and stopped in 3 (15%), methotrexate in 22 cases (54%) and stopped in 9 (41%), cyclosporine in 6 (15%) and stopped in 3 (50%), tacrolimus in 2 (5%), azathioprine in 11 (27%) and stopped in 4 (36%), mycophenolate mofetil in 6 (15%) and stopped in 2 (33%), rituximab in 7 (17%), IVIG in 6 (15%) and stopped in 1 (17%).

All but one patient referring to "ILD" group (n=76) were treated with corticosteroid (75/76; 99%), that were administered as IV bolus (methylprednisolone 1 g/day for 3 consecutive days) in 26 cases (35%). Of note, in 5 out of these 26 patients (19%), the bolus was administered due to muscle disease activity and not to ILD. Hydroxychloroquine was prescribed in 23 cases (30%) and stopped in 10 (43%), methotrexate in 19 cases (25%) and stopped in 10 (53%), cyclosporine in 23 (30%) and stopped in 12 (52%), tacrolimus in 8 (11%) and stopped in 3 (37%), azathioprine in 18 (24%) and stopped in 11 (61%), mycophenolate mofetil in 29 (23%) and stopped in 7 (24%), cyclophosphamide in 21 (28%) and stopped in 2 (10%), rituximab in 19 (25%) and stopped in 1 (5%), abatacept was prescribed in 1 case (1%), IVIG in 14 (18%) and stopped in 1 (7%). Drugs

were considered as stopped only if the withdrawal was due to infectiveness or side effects. The majority of patients received more than one immunosuppressant during the follow-up, even in association.

Conclusions
To the best of our knowledge, this is the largest anti-MDA5 positive cohort ever collected and one of the few describing a non-Asian setting. In analogy with previous reports (8-15, 22), we observed that anti-MDA5 antibodies were associated with a relatively high frequency of RP-ILD and mortality rate. In our cohort, 21% of patients had RP-ILD, whereas in Asian patients it is reported in up to 75% of cases (23), with the lower prevalence (33%) observed in a recently described Indian cohort of 25 patients (24). This heterogeneity might have accounted for the
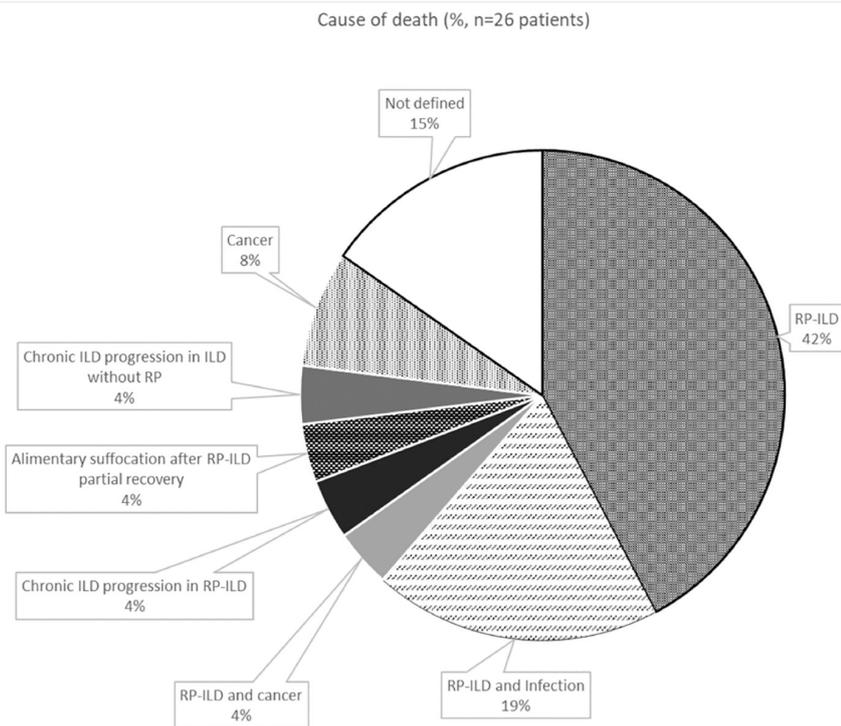
Fig. 4. Cause of death in the overall cohort.
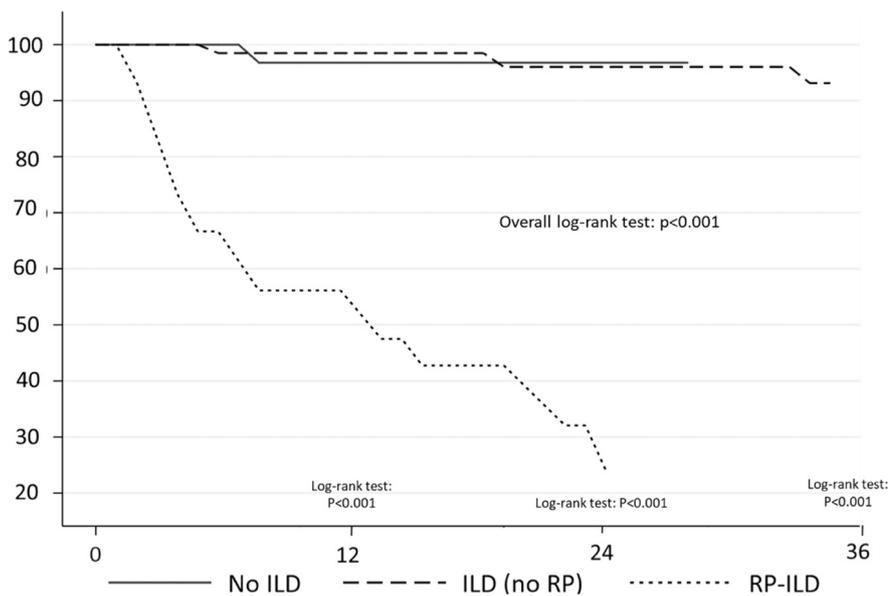ILD: interstitial lung disease; RP: rapidly progressive.

Fig. 5. Kaplan-Meier survival curves and Log-Rank test *p*-values (overall, and considering a cut-off time of 12, 24 and 36 months).
ILD: interstitial lung disease; RP: rapidly progressive.

In most of the cases arthritis, myositis, and cutaneous findings were observed at diagnosis and they may be considered a useful warning signal for the suspicion of this syndrome and an indication for the anti-MDA5 antibodies search in ILD patients (25). Our results are partially in contrast with a recent French report on 19 ICU admitted patients (18), describing an exclusive lung involvement in about one-third of cases. However, this study did not report how frequently these patients performed a complete rheumatological evaluation. This is not a secondary issue, since CTDs signs are not always easily detectable (25-27). Furthermore, as reported in a recently described US cohort (16), muscle involvement in our patients was mainly symptomatic or subclinical, in contrast to the Asian case series (15, 24, 29, 30) which reported mainly amyopathic diseases. Muscle involvement seems to be not so rare also in another recently described French cohort, with one-third of the cases presenting proximal muscle weakness, and about the 50% with increased CK levels (19), as a further confirmation of the possible occurrence of ethnicity related clinical phenotype differences and polymorphisms in HLA of anti-MDA5 antibodies positive patients. Interestingly, the hierarchical analysis of patients enclosed in this study identified 3 groups at different prognoses, based on the occurrence of RP-ILD (worst prognosis), dermo-rheumatology findings (good prognosis), and male patients with severe skin vasculopathy, and myositis signs (intermediate prognosis). In our cohort, the prognosis was influenced only by RP-ILD occurrence, whereas other patients had substantially a good prognosis, without substantial difference in the prevalence of other clinical findings. Furthermore, the different timing of occurrence of main clinical findings we showed leads the patients to a possible change of cluster during the follow-up.

A large number of patients fulfilled the ACR/EULAR Classification Criteria for Rheumatoid Arthritis (21), but only a few were classified as having an overlap with RA, indicating that joint involvement is only scarcely considered in these patients, even in case of typical

lack of a shared definition of RP-ILD (8-15). Surely, our definition was more stringent, since we aimed to avoid selection bias linked to the retrospective and multicentre nature of the study. However, it is also possible that the genetic background of the evaluated populations, not including Asian patients,

might have accounted for a lower prevalence of RP-ILD and thus for a lower rate of overall mortality. This hypothesis is supported also by the similar prevalence of RP-ILD (18%) observed in a recent French study which, however, included some patients of Asian ancestry (19).

Table II. therapies administered in RP-ILD patients, during ICU admission, stratified according to the outcome (alive after hospital discharge, and without subsequent ILD progression, or death during hospitalisation of for ILD subsequent progression), and to therapies performed in the 6 months previous ICU admission.

RP-ILD	Number (%)	Steroid pulses (1 g/day for 3-5 days, IV)	Steroid (PO)	Cyclosporine (PO)	Micophenolate mofetil (PO)	Cyclophos-phamide (IV)	Rituximab (IV)	IVIG	ECMO	No immuno-suppressive treatment
In ICU (alive)	13 (41)	5 (16)	1 (3)	1 (3)	5 (16)	5 (16)	5 (16)	1 (3)	2 (6)	0 (0)
In ICU (**death**)*	19 (59)	13 (41)	0 (0)	7 (22)	1 (3)	9 (28)	5 (16)	7 (22)	4 (13)	1 (3)
In the 6 months before ICU admission	28 (87%)	4 (13)	6 (19)	6 (19)	6 (19)	6 (19)	6 (19)	6 (19)	0 (0)	3 (9.5%)

ICU: intensive care unit; MMF: mycophenolate mofetil; RTX: rituximab; IVIG: intravenous immunoglobulins; ECMO: extracorporeal membrane oxygenation; PDN: prednisone; IV: intravenous; PO: per os.

clinical presentation. Interestingly, the clinical spectrum time course we observed in anti-MDA5 positive patients was quite similar to that of ASSD (1, 31-33), characterised by the occurrence of *ex-novo* findings in the 50% of cases, in line with a previous report (14). Approximately a third of our anti-MDA5 positive patients were concomitantly positive for anti-Ro52 kDA antibodies. A similar percentage was completely negative for baseline autoimmune profile generally applied in the screening of ILD (ANA test, including cytoplasmic positivity, anti-ENA antibodies, Rheumatoid factor, and anti-cyclic citrullinated peptide antibodies) (14), thus indicating that the suspect of the anti-MDA5 syndrome should be considered even in patients with a completely negative autoimmune profile. ANA assessment was performed with IIF but its execution in different laboratories and the use of different kits may have partially affected the results.

NSIP was the most frequent HRCT pattern, observed in about 2/3 of cases, not rarely associated with OP, in line with previous reports on MDA5 patients (34). Interestingly 14 patients (13%) had a UIP-like pattern, indicating that clinicians should consider anti-MDA5 antibodies occurrence independently to the underlying ILD pattern. FVC and DLCO were not different in both groups with lung involvement, but it was not possible to collect baseline PFTs in some RP-ILD cases for the very acute onset of respiratory failure. Furthermore, in consideration of the very highly variable length of follow-up, we were not able to compare PFT evolution in ILD subgroups.

Interestingly, ICU admission occurred generally early during the ILD course, as observed also in other studies (19), suggesting that the first months after lung involvement occurrence are at higher risk for RP-ILD, and require a close follow-up. In particular, we observed that ICU admission was more common in the first 12 months from ILD onset. We think that a strict multidisciplinary follow-up of anti-MDA5 positive patients is mandatory at least in the first 12 months from ILD onset, as the 84% of RP-ILD occurred during this timeframe.

Fever and lymphopenia were other common findings, in particular in RP-ILD patients. Fever may be a confounding factor, especially when the baseline autoimmune screening is negative: in such cases, the suspicion of infection may delay the immunosuppressive treatment. On the other hand, although previously included in the RP-ILD risk factors (11), we cannot exclude that lymphopenia in our cohort could be sometimes drug- and not disease-related. However, a pathogenetic link between lymphopenia and RP-ILD could be found by suggesting a possible role of viral infections in triggering RP-ILD, also considering the well-known antiviral function of MDA5 (35,36). Although we confirmed increased ferritinaemia levels in RP-ILD patients (36, 37), we were not able to determine its relevance in other groups. In analogy with previous reports, the outcome of our RP-ILD patients was unfavourable. To this purpose, many patients developed RP-ILD despite previous strong and multiple immunosuppressive therapies, including mycophenolate mofetil,

and rituximab, the drugs with the better performance in ICU (Table II), thus underlying the need for better RP-ILD risk stratification and treatment definition. Of note, none of the patients with RP-ILD we included in the study, even those on ECMO, was treated with plasmapheresis. Between the treatments performed in non-RP-ILD patients, mycophenolate mofetil, rituximab and IVIG had the lower rate of withdrawal. In one patient, lung transplantation was not effective, and the patient died for infectious complications of the immunosuppressive therapy.

We also showed that anti-Ro52 kDa antibodies positivity is more commonly observed in RP-ILD patients, thus confirming the prognostic impact of these antibodies also in non-Asian settings (39). On the contrary, we did not confirm the prognostic role of arthritis and RP (19), which were equally distributed between the 3 established groups.

Lastly, the relationship between anti-MDA5 antibodies and cancer must be clarified, since, in our retrospective study, this association was found only in a few patients unlike what was recently described in a Spanish case series (8), but in line with another recent French study (19). This discrepancy might at least in part have been explained by the lack of a systematic search for occult neoplasia in our series, in particular in those RP-ILD patients who had an unfavourable outcome. We cannot exclude that in these cases treatment refractoriness could have been related to occult neoplasia.

The main limitation of our study is its retrospective design, which entails the risk of missing data and heterogeneity

of clinical, therapeutic, functional, and laboratory measures.

On the other hand, given the rarity of this disease, retrospective multicentre studies are a necessary starting point.

Another limitation is the collection of data from different centres, which may have jeopardised the reliability of data. On the other hand, due to the rarity of the disease, the collaboration of numerous sites is necessary to recruit a significant number of patients. To homogenise the results among different centres, the same commercial kit for detection of anti-MDA5 antibodies was used and enrolment of patients was allowed only in tertiary centres.

In conclusion, we can confirm the necessity of a multidisciplinary approach and the search for rare myositis-specific antibodies in patients with ILD, even in the case of rapidly progressive pulmonary disease (40). We can also stress the need to rule out the presence of occult neoplasia and possible infective trigger in these patients since they might influence response to aggressive immunosuppressive regimens. Shared expertise between Rheumatologists, Pulmonologists, and ICU specialists is the only possible way to deal with such a burdening condition and it is the first necessary step for the identification of the best treatment options to be applied in these patients.

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