Prevalence and clinical significance of anti-MDA5 antibodies in European patients with polymyositis/dermatomyositis

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Abstract

Objective

Polymyositis/dermatomyositis (PM/DM) is an autoimmune disease characterised by skin and muscle inflammation, internal organ involvement and serum disease-specific autoantibodies. The recently identified anti-MDA5 (melanoma differentiation-associated gene 5) antibodies are associated with clinically amyopathic DM (CADM), rapidly progressive interstitial lung disease, severe skin manifestations, and poor prognosis.

Our objective was to examine the clinical significance of anti-MDA5 antibodies in a cohort of European Caucasian patients with PM/DM, considering that data on anti-MDA5 serology are limited to Asian and US cohorts.

Methods

Sera from 76 consecutive adult Italian patients with PM/DM were analysed by immunoprecipitation (IP) of ^{35}S-methionine radiolabelled HeLa and K562 cell extracts, ELISA using recombinant MDA5 protein and IP-Western Blot using rabbit anti-MDA5 antibodies. Clinical associations of anti-MDA5 antibody positive patients were analysed.

Results

Anti-MDA5 antibodies were identified in 5/76 (7%) PM/DM cases and all 5 cases were CADM; anti-MDA5 was the second most common autoantibody in DM after anti-MJ/NXP-2, found in 24% of cases. Compared to 29 anti-MDA5 (-) DM, anti-MDA5 (+) patients have more typical DM skin disease (digit pulp/periungual lesions, Gottron's papules, heliotrope rash) (p=ns). Interstitial lung disease was observed in 3/5 anti-MDA5 (+) patients but only 14% of anti-MDA5 (-) cases (p=0.048).

Conclusion

Our study on European patients with PM/DM confirms that anti-MDA5 antibodies are not uncommon. All anti-MDA5 (+) cases are affected by CADM with typical skin disease, while rapidly progressive pulmonary involvement was diagnosed only in one case. Further studies in larger cohorts are necessary to define the clinical significance of anti-MDA5 antibodies in European PM/DM.

Key words

autoantibodies, MDA5, dermatomyositis, polymyositis, interstitial lung disease, skin ulcers.

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Introduction

Polymyositis (PM) and dermatomyositis (DM) are autoimmune diseases characterised by muscle inflammation, and in DM skin is also a target of inflammation. Internal organs can be involved in both diseases, leading to conditions such as interstitial lung disease (ILD), proximal esophageal hypomotility and difficulty in swallowing, and cardiac arrhythmias (1-3). Moreover, PM/DM is characterised by the presence of serum myositis-specific autoantibodies (MSAs), a helpful tool for diagnosis, monitoring and management of patients (1, 4, 5). In fact, autoantibodies are present in 50–80% of PM/DM patients, and they are mainly detected as anti-nuclear and/or anti-cytoplasmic pattern in immunofluorescence (6, 7). In the last two decades, several MSAs have been identified besides anti-Jo-1 (histidyl tRNA synthetase), and they include autoantibodies to other aminoacyl-tRNA-synthetases (such as anti-PL-7, -PL-12, -EJ, -OJ, -KS, -Ha, -Zo), -CADM140 (clinically amyopathic dermatomyositis 140kD)/MDA5 (melanoma differentiation-associated gene 5), -TIF (transcription intermediary factor)1gamma/alpha (p155/140) and -MJ/NXP-2 (8, 9). Each of these antibodies is associated with unique clinical features. They are useful in the management of patients, and they may help in monitoring the response to treatment even if they do not invariably decline after successful therapy (4, 10-12). Among these newly-identified antibodies, anti-MDA5 (previously known as anti-CADM140) are strongly associated with clinically amyopathic DM (CADM) (12, 13), rapidly progressive interstitial lung disease (RPILD), severe skin manifestations, and symmetric polyarthritis (14-18). However, these features were previously reported mainly in Asian (Japan, Korea, and China) PM/DM cohorts positive for anti-MDA5, with the exception of two American studies. It is not known whether European Caucasian PM/DM patients have this specificity with similar clinical features. Our aim is to identify anti-MDA5 antibodies and their clinical associations in a cohort of European Caucasian patients with PM/DM.

Materials and methods

Patients

Seventy-six consecutive adult European Caucasian patients with PM/DM were enrolled in our retrospective study in the Rheumatology Unit in Brescia (Spedali Civili) in 2010–2012. Only 2/76 (3%) of the patients were not of Italian descent and they were from Eastern Europe. Forty control sera were also included in the study, and they were healthy European Caucasian subjects matched for sex and with non-significantly different age. The criteria of Bohan and Peter for PM/DM (19, 20) and the criteria of Sontheimer for CADM (21) were used to classify patients. Clinical information was obtained from clinical charts. No overlap syndrome was included in order to have a homogeneous cohort of PM/DM patients. The study was approved by the Institutional Review Board of the hospital. This study meets, and is in compliance with, all ethical standards of medicine, and informed consent was obtained from all patients in accordance with the Helsinki Declaration of 1975/83.

Interstitial lung disease (ILD) was defined on the basis of ground glass opacities documented by HRCT. Rapidly progressive ILD (RP-ILD) was defined as a condition of worsening radiologic interstitial change with progressive dyspnea and hypoxaemia within 1 month of the onset of respiratory symptoms.

Indirect immunofluorescence (IIF)

Immunofluorescent antinuclear/cytoplasmic antibodies (HEp-2 antinuclear antibody (ANA) slides, INOVA Diagnostics, San Diego, CA, USA) were tested using a 1:80 dilution of human sera followed by DyLight 488 donkey IgG F(ab)'2 anti-human IgG (Jackson ImmunoResearch, West Grove, PA, USA) (22).

Immunoprecipitation (IP)

Sera were analysed by immunoprecipitation (IP) of HeLa and K562 cell extract radiolabelled with ^{35}S-methionine; specificities of autoantibodies were determined by using specific reference sera (23).

Anti-MDA5 ELISA

Anti-MDA5 (+) antibodies were tested by ELISA, using recombinant MDA5 protein (0.5 microgram/ml, OriGene Technologies, Inc., Rockville, MD) and 1:250 diluted sera. Optical density was measured and converted into units using a standard curve created by a prototype positive serum.

IP-Western blot (IP-WB)

IP-Western blot (IP-WB) was performed as follows (24), using 2 microliter of human sera and cell extract from 5 x 10^6 HeLa cells. Proteins were fractionated by 7.5% SDS-PAGE, transferred to a nitrocellulose filter, probed using rabbit anti-MDA5 antibodies (AnaSpec, Fremont, CA) followed by horseradish peroxidase-conjugated goat anti-rabbit Ig light chain antibodies (Jackson Immunoresearch), and developed using Supersignal West Femto (ThermoFisher).

Statistical analysis

All parameters were analysed by Student's *t*-test, Mann-Whitney U-test, or Fisher's exact test between groups using Prism 6.0 for Windows (GraphPad Software Inc., La Jolla, CA). Statistical significance was accepted at $p<0.05$.

Results

Demographic data

Seventy-six patients (56 female and 20 male) were diagnosed with DM in 34 cases, PM in 30 cases and overlap syndrome in 12 cases. Based on the Bohan and Peter criteria (19, 20), the number of DM patients with definite diagnosis was 23, probable in 4 and possible in 7. Using the same criteria, we defined 13 PM patients as definite, 11 as probable and 6 as possible. However, all the PM/DM diagnosis of our cohort were confirmed based on the more recent Targoff criteria (7). The mean age of the whole cohort was 55.2 years (± SD14.5), the age at disease onset was 44.5 years (± SD18) and the median follow-up duration was 50 months (range 3–372). Gender ratio and age were similar in both groups (Table I).

Autoantibodies

Anti-MDA5 antibodies were identified in 5/76 (7%) PM/DM cases and they were the second most common specificity in DM after anti-MJ, as shown in Table I. When considering the 34 DM cases of our cohort, anti-MDA5 was found in 15% (5/34) and other autoantibodies identified were: anti-MJ (8/34, 24%), -p155/140 (4/34, 12%), -EJ (2/34, 6%), -Mi-2 (2/34, 6%), -PM/Scl (3/34, 9%) and -U1RNP (2/34, 6%). However, 24% (8/34) DM and 25% (19/76) in the whole PM/DM cohort still have no known myositis antibodies.

All the 76 sera were first tested by IIF, but we did not identify a specific immunofluorescence pattern associated with anti-MDA5 antibodies (data not shown). Then we performed anti-

Table I. Prevalence of autoantibodies in European Caucasian PM/DM patients.

	Total* n=76	DM n=34	PM n=30	*p*-value
Female	74% (56/76)	68% (23/34)	77% (23/30)	NS
Mean age, years (±SD)	55.2 (±14.5)	53.5 (±16)	55.3 (±14.7)	NS
Autoantibodies**				
MJ	11% (8/76)	24% (8/34)[1]	0% (0/30)[1]	0.005[1]
MDA5	7% (5/76)	15% (5/34)[2]	0% (0/30)[2]	0.05[2]
Jo-1	7% (5/76)	0% (0/34)[3]	17% (5/30)[3]	0.02[3]
p155/140	7% (5/76)	12% (4/34)	3% (1/30)	NS
SRP	4% (3/76)	0% (0/34)	10% (3/30)	NS
EJ	4% (3/76)	6% (2/34)	3% (1/30)	NS
Mi-2	4% (3/76)	6% (2/34)	3% (1/30)	NS
OJ	3% (2/76)	0% (0/34)	7% (2/30)	NS
PM/Scl	8% (6/76)	9% (3/34)	10% (3/30)	NS
U1RNP	5% (4/76)	6% (2/34)	7% (2/30)	NS
No known MSAs	25% (19/76)	24% (8/34)	37% (11/30)	NS

*Overlap cases (n=12) are not shown and analysed in the table; **Some patients having more than one specificity are not shown in the table.

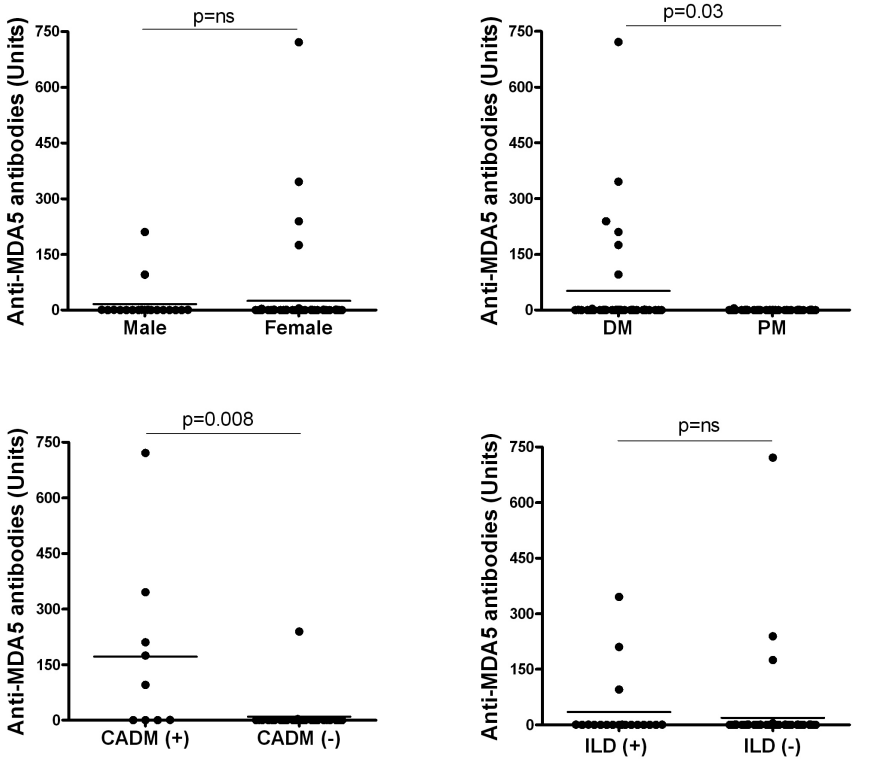

Fig. 1. Anti-MDA5 antibodies in adult European PM/DM patients by ELISA. Anti-MDA5 antibodies in 1:250 diluted sera were tested by ELISA using recombinant MDA5 antigen. Results are shown in Units, and the median is represented. $p<0,05$ by non-parametric Mann-Whitney test.

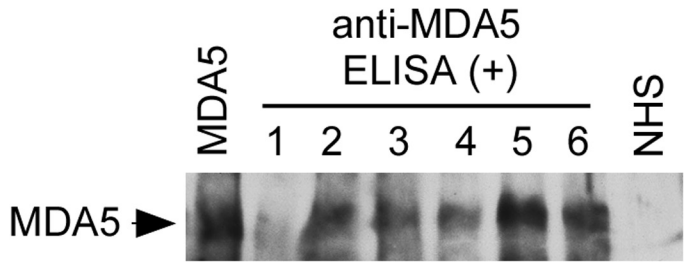

Fig. 2. IP-WB of anti-MDA5 ELISA positive sera. HeLa cell extract was immunoprecipitated by anti-MDA5 prototype serum (lane MDA5), anti-MDA5 ELISA positive European Caucasian sera (lanes 1-6; lane #3 is the patient of African origin not included in the analysis of clinical parameters) and normal human serum (NHS), and probed with rabbit anti-MDA5 antibodies.

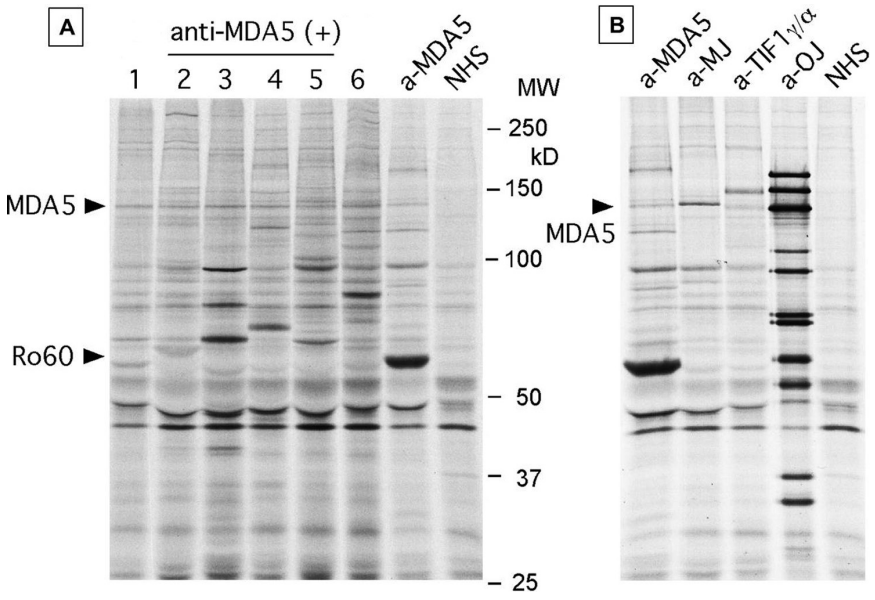

Fig. 3. IP of anti-MDA5 ELISA-positive sera. ^{35}S-methionine labelled HeLa cell extract was immunoprecipitated by human sera and analysed by 8% SDS PAGE. Anti-MDA5 antibodies immunoprecipitate a ~140kD protein (arrow in panel A and B; the anti-MDA5 standard is known to have strong reactivity to Ro60, as shown by the arrow in panel A; lane #3 is a patient of African origin not included in the analysis of clinical parameters) that is different from the proteins immunoprecipitated by other reference sera (panel B: anti-MJ, anti-TIF1gamma/alpha, anti-OJ; NHS: normal human serum).

MDA5 ELISA for screening, and we found 6 anti-MDA5 (+) sera at high levels (mean Units 298 ±SD223; Fig. 1). The specificity of all 6 ELISA positive sera was then confirmed by IP-WB (Fig. 2) and also by IP of HeLa cell extract (Fig. 3). One anti-MDA5 (+) shown in Figures 2 and 3 was not included in our data analysis because the patient was not European Caucasian but African.

The 40 controls were healthy subjects matched for sex to the patients, their age was not significantly different from the patients, and they did not show any positive autoantibody specificity in our analysis.

The K562 cell extract was not used for anti-MDA5 identification because the expression of MDA5 antigen was poor (24). The MDA5 antigen of ~140kD is visible by IP in our 6 positive DM samples (Fig. 3, panel A) when HeLa cell extract was used. The MDA5 antigen comigrates very close to the MJ/NXP-2 antigen, as shown in Figure 3 panel B. Other PM/DM autoantibodies such as anti-TIF1 gamma/alpha and -OJ detect an antigenic component of about 140 kD. The distinction of these different specificities requires the use of different methods, and this is the reason why we used ELISA, IP-WB and IP for the definition of each autoantibody specificity (Fig. 3, panel B).

Clinical and laboratory features

All the 5 anti-MDA5 (+) patients had a diagnosis of DM, and none had an overlap syndrome or PM. The main features of 5 anti-MDA5 (+) DM cases were analysed vs 29 anti-MDA5 (-) DM, as shown in Table II. No significant difference was observed in the two groups for sex ratio and mean age. Despite observing more typical DM skin features in anti-MDA5 (+) patients, such as skin ulcers (digit pulp/periungual), Gottron's lesions and heliotrope rash (60% *vs*. 55%), compared to anti-MDA5 (-) DM, the difference did not reach statistical significance (Table II). No other DM cutaneous or mucosal feature was significantly different between the two groups.

As for internal organ involvement, we focused on lung inflammation because severe ILD is the main cause of death and poor prognosis in patients with anti-MDA5 (+) PM/DM. Also in our cohort, ILD is more common in the anti-MDA5 (+) DM patients (60%) *versus* anti-MDA5 (-) (14%; p=0.048): two patients underwent high dose steroid plus intravenous cyclophosphamide therapy with resolution of alveolitis, and one died due to RPILD. One also developed subcutaneous emphysema with pneumomediastinum. We observed a lower average percentage of DLCO in the anti-MDA5 (+) compared to anti-MDA5 (-) patients (66% *vs*. 78%), though not statistically significant. In the anti-MDA5 (-) DM group, only one patient did not solve alveolitis and later developed pulmonary fibrosis. Immunosuppressive treatments used in anti-MDA5 (+) and (-) DM patients are shown in Table II, and the outcome expressed by mortality cases was similar between the two groups, with only one patient deceased for RP-ILD in anti-MDA5 (+) DM. When considering other features of systemic involvement, such as arthritis, Raynaud's phenomenon, oral ulcers, calcinosis and malignancy, we did not identify a significant trend or association with anti-MDA5 antibodies. As for muscle inflammation, also in our cohort all the anti-MDA5 (+) DM patients had CADM (*vs*. 17% in anti-MDA5 (-) DM; p=0.0009).

Table II. Demographic and clinical features in anti-MDA5 (+) and -MDA5 (-) European Caucasian DM.

	DM anti-MDA5 (+) n=5	DM anti-MDA5 (-) n=29	*p*-value
Demographic data			
Female	60% (3/5)	66% (19/29)	NS
Mean age, years (±SD)	54 (±13)	53 (±16)	NS
Clinical data			
Mean DM duration, before amyopathic DM onset	1 year (range 1-4)	1 year (range 0-2)	NS
Amyopathic DM	100% (5/5)	17% (5/29)	0.0009
Median CPK at onset (range)	55.5 (44-102)	1600 (18-16490)	0.02
Skin disease			
Facial erythema	80% (4/5)	59% (17/29)	NS
Heliotrope rash	60% (3/5)	55% (16/29)	NS
Gottron's papules	40% (2/5)	48% (14/29)	NS
Skin ulcerations	20% (1/5)	7% (2/29)	NS
Gottron's + skin ulcerations	60% (3/5)	55% (16/29)	NS
Lung involvement			
ILD	60% (3/5)	14% (4/29)	0.048
Mean DLCO,% (±SD)	66 (±26.2)	78 (±18.5)	NS
Arthritis	0	10% (3/29)	NS
Raynaud	0	34% (10/29)	NS
Cancer	0	10% (3/29)	NS
Treatments used as first, second or third option	steroid, methotrexate, cyclosporine, hydroxychloroquine, azathioprine, yclophosphamide, IV immunoglobulins	steroid, methotrexate, cyclosporine, hydroxychloroquine, azathioprine, cyclophosphamide, IV immunoglobulins, mycophenolate	–

Discussion

Most previous reports on anti-MDA5 antibodies studied cohorts of DM patients in Asian countries, mainly Japan, China and Korea (18, 25-27), except for some recent studies from the United States (14, 15), but not from European Caucasian cohorts (Table III). These reports describe the main clinical features of anti-MDA5 positive DM patients, such as CADM (12, 13, 28), high prevalence of RPILD leading to poor prognosis (29), and in one of the two cohorts of American DM patients, the authors also identified a unique cutaneous phenotype characterised by skin ulcerations, tender palmar papules, or both, and by severe arthritis (14, 15). The pooled sensitivity and specificity of anti-MDA5 antibodies to predict RP-ILD in patients affected by PM/DM are very good, and they are estimated to be 77% and 86%, respectively (16). However, prevalence and specificity of autoantibodies in PM/DM are quite different between studies from different countries or even within the countries (30-32), and this is true also for anti-MDA5 antibodies, as shown in Table III. Studies on prevalence of anti-MDA5 in DM showed a prevalence ranging from 3% to 58%, and this percentage increases up to 100% when only CADM patients are considered (Table III). Also, prevalence of MSAs in Italian patients is very different from the one reported in Caucasians from the United States, UK and other European countries; in particular, prevalence of anti-MJ was very high in our Italian cohort (24). Thus, it is important to accumulate data on prevalence and clinical association of MSAs from different ethnicity (32). Our study is the first report on anti-MDA5 antibodies in adult European Caucasian patients with PM/DM.

We identified anti-MDA5 antibodies in 7% (5/76) of the whole cohort, and they were the second most frequent specificity after anti-MJ antibodies (8/76, 11%). All 5 anti-MDA5 (+) patients had a diagnosis of CADM with normal CPK levels similarly to reports from other countries (16), and a significantly higher prevalence of ILD compared to anti-MDA5 (-) DM patients. We completed the analysis of clinical features of the whole cohort, but we did not find other significant differences in clinical aspects.

From a laboratory point of view, most myositis autoantibodies are detected by IP using K562 cell extract, but MDA5 expression in K562 cells is variable and often weak or absent (24). Thus, ^{35}S-methionine labelled HeLa cell extract, which expresses MDA5, was used for IP-WB and IP to confirm anti-MDA5 (+) samples identified by ELISA with recombinant MDA5 antigen. IP is a powerful technique to identify various MSAs by a single assay, although it is technically demanding and for this reason it is used only in a limited number of institutes. It is generally difficult to confirm the specificity of an uncharacteristic single protein autoantigen since many proteins have a similar or identical mobility on SDS-PAGE and IP. Anti-MDA5 is one of these autoantibodies because many known and unknown autoantigenic proteins have a molecular weight around 140kD as the MDA5 antigen, and that is why methods alternative to IP are required for confirmation (14, 15, 25). Anti-MDA5 ELISA is useful in screening PM/DM samples thanks to the commercial availability of recombinant MDA5 proteins (14, 16), and in fact in our study all the 5 anti-MDA5 (+) ELISA samples were confirmed by IP and IP-WB.

In summary, anti-MDA5 antibodies are the second most common specificity in our cohort of adult European Caucasian DM patients, beside anti-MJ antibodies already described (24). Anti-MDA5 (+) patients are affected by DM with specific features such as CADM, RPILD and skin ulcerations. IP of HeLa cell extract, IP-WB and anti-MDA5 ELISA are the methods suggested for anti-MDA5 identification. We acknowledge the limitations of our study, mainly the small cohort of PM/DM patients and the retrospective collection of clinical information, so further studies in larger cohorts and with patients from different ethnic groups are necessary to better define the clinical significance of anti-MDA5 antibodies in European PM/DM patients.

Table III. Prevalence of anti-MDA5 antibodies in the literature.

Reference	Present study	(33)	(34)	(23)	(29)	(14)	(14)	(26)	(28)	(13)	(21)	(15)	(16)
Year	2013	2005	2009	2010	2010	2011	2011	2012	2012	2013	2013	2013	2013
Country	Italy	Japan	Japan	Japan	Japan	Japan	USA	China	Japan	Japan	Japan	USA	China
Department	Rheum.	Rheum.	Rheum.	Rheum.	Derm.	Derm.	Derm.	Rheum.	Derm.	Rheum.	Derm.	Rheum.	Rheum.
n. (PM/DM)	76	103	120	-	88	-	-	113	-	-	-	-	64
n. (DM)	34	42	35	24	82	95	77	76	51	63	95	160	34
n. (CADM)	5	15	32	15	31	36	13	8	30	17	22	11	9
% of DM in PM/DM	34/76 =45%	42/103 =41%	35/120 =29%	-	82/88 =93%	-	-	76/113 =67%	-	-	-	-	34/64 =53%
% of CADM in PM/DM	5/76 =7%	15/103 =15%	32/120 =27%	-	31/88 =35%	-	-	8/113 =7%	-	-	-	-	9/64 =14%
% of CADM in DM	5/34 =15%	15/42 =36%	32/35 =91%	15/24 =63%	31/82 =38%	36/95 =38%	13/77 =7%	8/76 =11%	30/51 =59%	17/63 =27%	22/95 =23%	3/160 =2%	9/34 =26.5%
Prevalence of anti-MDA5 in PM/DM	5/76 =7%	8/103 =8%	23/120 =19%	-	22/88 =25%	-	-	38/113 =34%	-	-	-	-	26/64 =41%
Prevalence of anti-MDA5 in DM	5/34 = 15%	8/42 =19%	1/35 =3%	14/24 =58%	21/82 =26%	26/95 =27%	5/77 =6.5%	14/76 =18%	31/60 =52%	14/63 =22%	25/95 =26%	11/160 =7%	17/34 =50%
Prevalence of anti-MDA5 in CADM	5/5 =100%	8/15 =53%	22/32 =69%	8/15 =53%	20/31 =65%	25/36 =69%	5/13 =39%	5/8 =62.5%	-	12/17 =71%	22/25 =88%	7/11 =64%	9/9 =100%

Rheum: Rheumatology; Derm: Dermatology.

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Note added

Recently an excellent publication on anti-MDA5 antibodies in the Mediterranean population was published:

LABRADOR-HORRILLO M, MARTINEZ MA, SELVA-O'CALLAGHAN A, TRALLERO-ARAGUAS E, BALADA E, VILARDELL-TARRES M, JUÁREZ C: Anti-MDA5 antibodies in a large Mediterranean population of adults with dermatomyositis. *J Immunol Res* 2014; 2014: 290797 [Epub 2014 Feb 4].

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