ORIGINAL ARTICLE

Prevalence and Clinical Correlates of Myositis Specific Autoantibodies in Idiopathic Immune-Mediated Inflammatory Myositis - Results from a Multicentric Cohort (MyoIN) from India

Liza Rajasekhar[1*], Vineeta Shobha[2], Anitha Narasimhan[3], Vasudha Bhat[4], SN Amin[5], Ramnath Misra[6]

Abstract

There is a need to understand the clinical and antibody associations in patients with IIM in various ethnicities and geographical areas. Patients who fulfilled Bohan's and Peter criteria of IIM and seen between October 2017 through Jan 2020 were enrolled in this study at 3 centres**.** Clinical and relevant laboratory parameters were recorded in a pre designed case record form. MSA and MAA to 16 antigens were performed by line blot assay using Euroimmun (Luebec, Germany) as per manufacturer's instruction. Of the 150 patients, 13 were juvenile onset. Ninety sera had either one MSA or MAA. Sixty sera had neither MSA/MAA. anti-Ro 52 were the commonest antibody and anti-Mi-2α and b the commonest MSA. Novel associations identified were severe myositis with anti-Ro 52, cutaneous ulcerations with anti-MDA5 and anti-PM-Scl and calcinosis with anti-PM-Scl. One-third sera had no MSA or MAA. Larger sample size and use of antibody assays together with muscle biopsy will improve subtyping and phenotype associations in IIM.

Introduction

Immune- mediated inflammatory myositis (IIM) is a heterogenous group of rare multisystem disorder predominantly affecting skeletal muscles and often affecting the skin, joints, and lungs. Their sera often have antibodies designated muscle-associated autoantibody (MAA) or muscle-specific autoantibody (MSA). Over the last three decades, these autoantibodies are being increasingly used to classify IIM into phenotypic and prognostic subgroups and to predict outcome or therapeutic response. Substantial overlap exists between various clinical phenotypes and autoantibody presence. Earlier, their detection methods were complex and were time and labour consuming such as immunoprecipitation and immunodiffusion. With the availability of ELISA and line immunoblot assays, MAA/ MSA are being increasingly used in day-to-day clinical practice. Yet, the recent EULAR/ACR classification for criteria for adult and juvenile IIM[1], could use only anti Jo-1 as a criteria since not enough data was available for other autoantibodies. There is uncertainty whether MSA/ MAA assays would truly prove to be useful as diagnostic or prognostic tools in the long term. Presence of these autoantibodies in the normal and the non-IIM population and determination of appropriate cut-offs to balance sensitivity and specificity also needs attention.

Genetic and or environmental factors impact the clinical phenotype and the autoantibody distribution of IIM[2]. There is a difference of opinion if these MSA/MAAs are truly mutually exclusive, and that the distinct or unique clinical features are probably not sacrosanct. There is only one published report from North India of MSA and MAA occurrence in patients with India which studied earlier targets (Jo-1, PL-7, PL-12, Mi-2, SRP) with increased prevalence of antibodies to Mi-2 as DM was the predominant subtype. There is an urgent need to clarify the extent and scope of clinico-serologic association in IIM and to establish its prevalence and consistency in various ethnicities and geographic regions across the world. Current study was undertaken to describe the prevalence of extended profile MSA and MAAs in IIM cohorts collected from across India and to study their clinical associations..

Patients and Methods

Patients who fulfilled Bohan's and Peter criteria of IIM and seen between October 2017 through Jan 2020 were enrolled in this study at 3 centres, two of them tertiary teaching referral centres and one specialist clinic. Consecutive patients were enrolled into MyoIn cohort[3] as inception cohort (newly diagnosed) or prevalent cohort. Overlap with connective tissue disease and cancer associated myositis were considered if they met their respective clinical criteria. Those with muscular dystrophy, metabolic myopathy or neurogenic myopathy were excluded. Clinical and relevant laboratory parameters were recorded in a pre-designed case record form and data was entered into the Microsoft

[1]Professor, Department of Clinical Immunology and Rheumatology, Nizam's Institute of Medical Sciences, Hyderabad, Telangana; [2]Professor, Department of Clinical immunology and Rheumatology, St John's Medical College Hospital, Bangalore, Karnataka; [3]Research Co-ordinator, Department of Clinical immunology and Rheumatology, Nizam's Institute of Medical Sciences, Hyderabad, Telangana; [4]Research Fellow, Department of Clinical immunology and Rheumatology, St John's Medical College Hospital, Bangalore, Karnataka; [5]Consultant, Rheumatic Disease Clinic Mumbai, Mumbai, Maharashtra; [6]Professor and Head Clinical Immunology and Rheumatology Kalinga Institute of Medical Sciences, Bhubaneswar, Orissa; [*]Corresponding Author

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Table 1: Demographic and clinical characteristics of patients with IIM

	Total	DM +ASS	Overlaps	PM	Others	Juvenile myositis
Number	150	87	42	17	4	13
Female: Male	(111;39)	(60: 27)	(38:4)	(11:6)	(2:2)	(9:4)
Age, mean (SD) years	40.74(14.84)	39.81(15.56)	37.80(11.96)	50.76(14.42)	49.17(11.40)	12.56(5.42)
Disease duration, mean (SD) years	0.78(1.8)	0.51(0.93)	1.28(2.90)	0.99(1.66)	0.31(0.46)	0.37(0.54)
Duration of follow-up, mean (SD) years	3.48(4.7)	3.03(4.56)	3.10(3.84)	7.48(6.42)	0.20(0.28)	1.01(1.52)
Severe myositis at presentation	115	63	26	13	3	12
Pharyngeal weakness	49	27	13	7	2	2
Respiratory muscle weakness	25	17	5	3	0	0
Typical DM rashes	60	53	5	0	2	7
Antisynthetase features (Fever/ Mechanic's hand/ arthritis	106	64	32	7	3	11
anti-Jo -1	15	15				1
anti-(PL-7, PL-12, EJ, OJ)	6	5	1			
anti-(Mi-2α, Mi-2 β)	17	12	3		2	2
anti-SRP	4	2	2			
anti-MDA5	7	6	1			1
anti-TIF-1γ	7	6	1			1
anti-NXP-2	6	5	1			2
anti-Ro 52	46	28	16	2		1
anti-PM-Scl75, PM-Scl100	12	7	4		1	1
anti-Ku	8	2	4	2		1

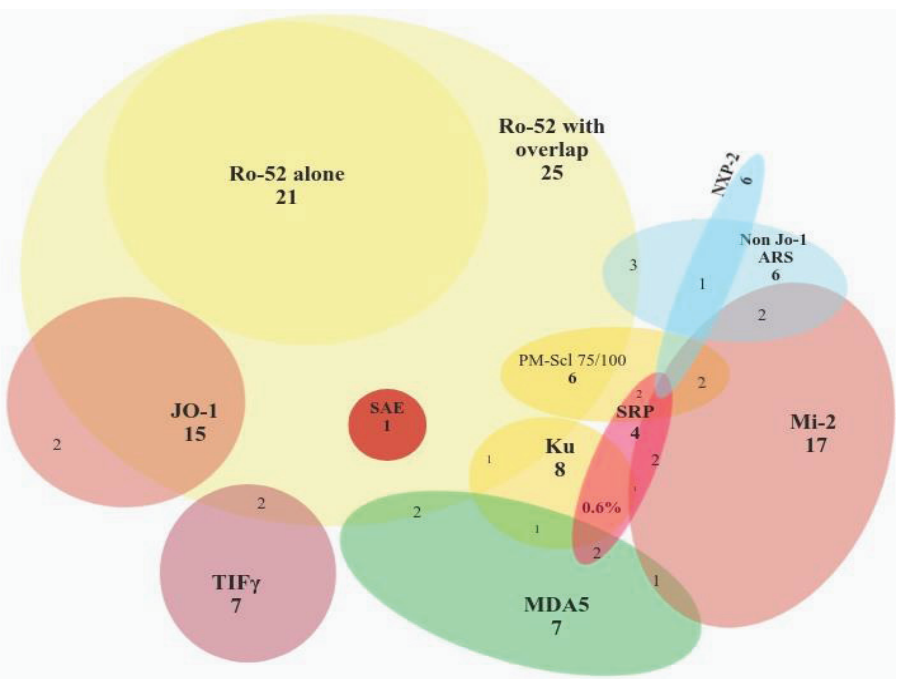

Fig. 1: Prevalence and overlap of MSA/MAAs (n=90/150)
Fig. 1: Frequency of MSA/MAA. Frequency of overlap with another antibody is mentioned at periphery of the circles

Access database developed for this purpose. Electromyography (EMG) was performed in all cases but muscle biopsy was undertaken as per physician's investigator discretion. Interstitial lung disease (ILD) was diagnosed based on HRCT and or restrictive physiology in presence of dry cough or dyspnoea. antinuclear antibody assay was performed by IIF on the Hep-2 cell line in 1:100 dilutions. MSA and MAA to 16 antigens were performed by line blot assay using Euroimmun (Luebec, Germany) as per manufacturer's instruction. These antigens were Mi-2α, Mi-2β, TIF-1γ, MDA5, NXP-2, SAE1, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ, Ro-52. Results were categorised as 0–5 (neg), 6-10 (borderline), 11–25 (+) and 26-50(++), strong positive (+++) respectively. In this study antibody positivity was defined by a blot intensity of 25 or more. Correlations between phenotypic features and antibodies and between various antibodies was performed using Pearson's correlation coefficient, in the SPSS version 20. Correlation of multiple antibodies with clinical features were done using the Kruskal Wallis test. Comparison of multiple group means with ANOVA.

Institutional ethics committee of each centre approved this study with all subjects or their legal representative providing written informed consent. The MSA EUROIMMUN line blot assay kits were generously provided by the Physician Research Foundation, academic wing of the Association of Physicians of India.

Results

Of the 150 patients, 63 were newly diagnosed (inception cohort) and 87 belonged to the prevalent cohort. These included 13 juvenile onset myositis. The ACR/EULAR score for classification as IIM[2] was applied to 137 adult participants. Sixteen of 137 (11%) adult patients could not meet the ACR/EULAR IIM score criteria for definite or probable myositis.

The demographics, clinical phenotype and MSA/MAA details of the150 patients are provided in Table 1.

Patients diagnosed with polymyositis were older with a longer duration of follow-up. The Venn diagram (Figure 1) shows the spread and overlap of the MSA/MAAs. Ninety sera had either one MSA or MAA. Sixty sera had neither MSA/MAA. Dual antibodies were identified in 30 patients and 4 had ≥3 autoantibodies.

There was a strong association between anti- Mi-2α, Mi-2β antibodies (p=0.002) and between anti-PM-Scl75 and 100 (p=0.00). antiMi-2α, Mi-2β, MDA5, NXP-2, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, Ro-52 all were significantly associated with more than one antibody in 2+ titres. Only anti- TIF-1γ, SAE1, EJ had no association with another antibody. Figure 2 shows the distribution of the intensities of each MSA/MAA.

Table 2 summarises the significant associations between a phenotype and antibodies.

As is evident from Table 2 associations of ILD with ARS, cutaneous ulcerations

	6-10	11-25	26-50	>50
PL-7	12	4	3	2
PL-12	11	6	0	0
EJ	1	0	1	0
OJ	2	1	0	0
Anti Jo-1	4	5	0	15
Anti Mi-2b	18	12	7	3
Anti Mi-2a	17	10	7	3
Anti-MDA5	7	4	4	3
Anti PM-Scl 75	8	5	0	6
Anti PM-Scl100	9	10	5	1
NXP-2	3	1	1	4
Anti SRP	11	15	2	2
Anti SAE	13	9	0	1
TIF Ig	6	2	4	3
Anti-RO 52	4	8	12	34
Anti Ku	13	16	6	2

Fig. 2: Intensity of all MSA/MAAs (%)

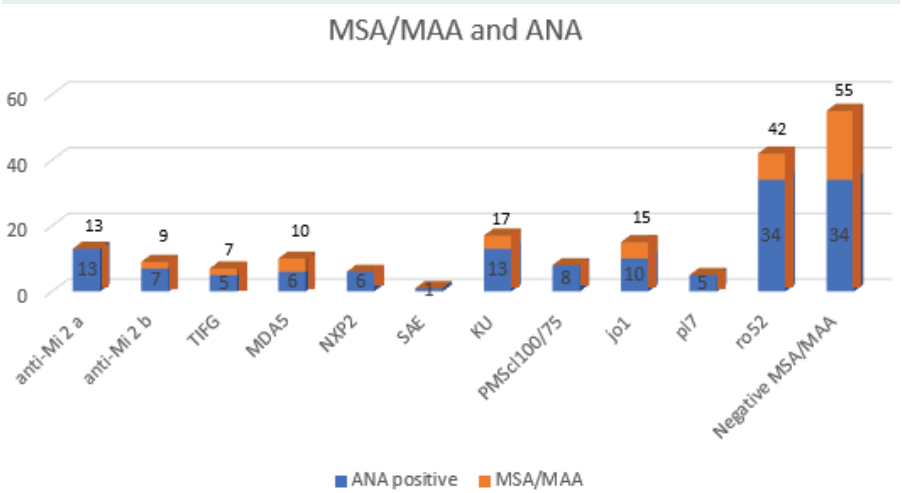

Fig. 3: ANA positivity in all MSA/MAAs (n= 105/136)

with anti-MDA5 and DM with anti-Mi-2α and anti-NXP-2 antibodies were confirmed. Dermatomyositis was associated with anti- Mi-2α, Mi-2β and NXP-2. Additionally, we discovered an association between dysphagia and anti-Mi-2β anti-TIF-1γ, anti-Jo-1. anti-Ro52 antibodies were associated with worse functional outcome. No association was found between ILD and MDA5. There were too few malignancies and anti-TIF-1γ to derive an association.

Correlation of ANA with MSA/MAA

ANA by IIF at a dilution of 1:100 with an intensity of 2+ and above was positive in 105 of 136 (77.2%). Only anti-Mi-2β correlated with ANA positivity (p=0.00). Cytoplasmic staining was noted in 20/105. In 55 sera with no MSA/MAA, 21 were negative for ANA as well. In the 34 with a positive ANA, the most frequent pattern was nuclear speckled (11/34). Cytoplasmic staining pattern was noted in almost a quarter (8/34).

Table 2: Significant associations of MSA/MAA with a clinical phenotype

DM specific rash	Antibody	p value
Gottron's papules	MDA5	0.016
	SAE1	0.005
Gottron's sign	MDA5	0.03
Heliotrope rash	TIF-1γ	0.00
DM	Mi-2 α	0.009
	NXP-2	0.09
Calcinosis	PM-Scl75	0.00
	EJ	0.001
Cutaneous ulceration	MDA5	0.02
	PM-Scl75	0.01
ILD	Jo-1	0.00
	PL-7	0.01
	Ro52	0.01
Dysphagia	Mi-2 β	0.01
	TIF-1γ	0.06
	Jo-1	0.001
Poor functional class at presentation	Ro-52	0.01

Table 3: Histopathological findings in subtypes of IIM

	DM+ASS	PM	OM	Total
Endomysial Inflammation	8	5	5	19
Perimysial inflammation	11	4	7	22
Perifascicular atrophy	17	5	5	27

MSA/MAA negative patients

In 60 patients with no antibodies 27 had DM with 20 having a DM specific rash. In 34 patients ANA were detected. Twenty five of these 60 subjects had undergone a muscle biopsy, 23 had unequivocal evidence of IIM on biopsy in this subset. In two, biopsy was not consistent with myositis. One of them had anti-Jo-1 positive done by ELISA elsewhere and the second patient had elevated CPK and a cytoplasmic speckled ANA with anti-Ro52 positive by ELISA and the rest.

Histopathology

Muscle biopsies were done in 62 patients. Table 3 lists the major findings in the various subtypes of IIM.

Discussion

There is increasing consensus among experts that MSA/MAA define relatively homogenous subsets of IIM[4]. We present cross-sectional multicentre data of MSA and MAA in 150 patients of IIM from India, using the 16Ag

Table 4: Comparison of autoantibody prevalence in various IIM cohorts

	MyoIn cohort, Pan Indian	Srivastava, North Indian	Platteel Dutch	Chen, Chinese cohort	Chen, Japanese cohort
N	150	124	187	145	165
Type of IIM subsets included	DM, OM, PM, ASS, CAM IMNM, IBM				
Age mean(yrs)	40	30.4	62	49.3	51.2
ANA pos (%)	77	68.9	ND	NA	NA
MSA/MAA (%)	60	73.4	47.1%/ 16.6%	cannot be compared	cannot be compared
Cut-off	intensity levels >25	moderate reactivity	intensity levels >25	IP ELISA (MDA5) Immunoblot	IP ELISA (MDA5) immunoblot
ARS (Jo-1 and Non Jo-1(%)	14 (10&4)	23(11&12)	18.2(10&8)	27.6	40
Mi-2 (%)	11	21	7.5	4.1	2.4
PM-Scl75/100(%)	5.3	14.5	12.4	ND	ND
Ro-52 (%)	30	36.3	NA (excluded)	ND	ND
TIF-1 y (%)	4.6	not reported	7.0	5.5	8.5
NXP-2 (%)	4	Not reported	2.1	4.8	3.6
MDA5(%)	4.6	not reported	5.4	36.6	15.8
SRP(%)	2.6	not reported	5.9	1.4	7.9
Ku (%)	5.3	10.5	4.3	0.7	0.6
2 antibody pos (%)	20	30	23/ 119		
Ro Jo correlation	r=0.41	r=0.31	Ro excluded	ND	ND

EUROIMMUN line-blot assay which includes the first reports of anti-TIF-1γ, MDA5, NXP-2, SAE1 antibodies. While giving an overview of distribution of MSA/MAA in IIM populations spread across the country, we also report a large subset of MSA/ MAA negative myositis patients, a strong association between anti-Ro 52 and anti-Jo-1, and demonstrate that apart from known association of ILD was associated with the anti-synthetase antibodies, anti-Ro-52 was also associated with ILD and severe myositis. Patients with anti MDA5 antibodies had cutaneous ulcerations as did those with anti-PM-Scl, the latter also associated with calcinosis. Of the 90 patients with atleast one antibody, in 38% we report the presence of more than antibodies in significant titres.

While Heliotrope rash was associated with anti-TIF-1γ antibodies, Gottron's papules and sign were associated with anti-MDA5 antibodies. Mechanic's hands were seen in patients with OM also and ARS antibodies were not more frequent than other antibodies in these patients. Cutaneous ulcerations were almost always associated with the presence of either MSA/MAA, and correlated with presence of anti-MDA5 and PM-Scl75. Of 75 DM patients 65% were MSA negative. We had very few patients with malignancy in our cohort to comment on the association of anti-TIF-1γ with malignancy.

Comparative data of MSA/MAA prevalence from various cohorts is presented in Table 4. The Indian and Dutch studies[5] used the Euroimmun platform however the study comparing the Chinese and Japanese population[6] used IP, IB and ELISA. Anti Ro-52 was the most prevalent antibody in our cohort either alone or in combination with anti Jo-1, which is similar to previous reports.

We report a low prevalence of anti-MDA5 positivity in our cohort. A much higher frequency of 36.6% has been mentioned in recent reports of DM from China, Japan[6] (15.8%) and Mediterranean population of DM (12%).[7] Prior reports from Japan, China, and Korea have demonstrated high mortality and poor prognosis in these cohorts. Despite CPK being almost normal, most of the patients in our cohort had poor functional class and a high mortality rate of 28.7%. As expected, cutaneous ulceration correlated with anti MDA5.

As in other cohorts, anti-Mi-2 antibodies were associated with a clinical diagnosis of DM. anti-Mi-2α had stronger correlation with diagnosis of DM than anti-Mi-2β, however both antibodies were tightly correlated. Clinical phenotype of anti-Mi-2 is described as classic DM skin rash, good response to steroid resulting in good prognosis. However, in our cohort, only half the patients had a DM specific rash, the majority had poor functional status and very high CPK levels. No association was found with ILD.

The prevalence of anti-aminoacyl t-RNA synthetase antibodies (ARS) of 14% is far lesser than the previously reported Indian cohort (23.4%),[8] the Euromyositis cohort [7](22.2%), Chinese (26.4%) and Japanese (40%) cohorts. It is pertinent to note that all three cohorts used immunoprecipitation, the Euromyositis cohort used [35S]-methionine labelled K562 cell extract, while the Chinese and Japanese cohorts used HELA cell lines. As is now well established, even in our cohort ILD was strongly associated with anti-Jo-1 and anti-PL-7 antibodies.

Polymyositis proportion was higher in the previous cohorts, probably attributable to prevalent understanding of IIM at that time. As we have learnt since the discovery of MSA and recognition of MAA and with the use of muscle biopsy findings of perimysial inflammation and perifascicular atrophy more and more PM can be classified either as DM, OM, IMNM or IBM.

The concept of overlap myositis is evolving from a myositis associated with an established disease like scleroderma or lupus to one where the myositis is accompanied by either a complete disease or an association with one of many clinical feature like Raynaud's phenomenon or an antibody which can be ANA or a myositis associated antibody like anti-Ro-52 or anti-PM-Scl etc[9]. Two third of our overlap myositis cohort consisted of scleromyositis. Six OM patients (2 SSc, 3 SLE and 1 is Sjogren's) had one or more MSAs (Mi -2 β, MDA5, SRP, NXP-2, TIF-1γ, PL-7 one each) suggesting that MSA can add value to clinical features to accurately categorise subsets.

In the MSA/MAA negative subset 11 of 25 who had a muscle biopsy had PFA, considered the hallmark of DM. This gives value to muscle biopsy as a tool in subclassification in autoantibody negative patients, at least till the time more antisynthetase or other novel antibodies are discovered or incorporated into assays.

There have been increasing concerns regarding ideal cut-offs and sensitivity for various autoantibodies in the 16Ag EUROIMMUN line-blot assay. Our

data (Figure 2) reveals many antibodies (anti-Mi-2α and β, PM-Scl100, anti SRP and anti-Ku were frequently (in more than 10 patients each) positive at lesser titres. In our cohort, 3 patients negative for MSA/MAA had ELISA positive for anti-Jo-1, anti Mi-2α and anti-Ro52. It has been reported and analysed by others[5,10] that we may need different cut-offs for the different antigens present in the line immunoblot.[8] As and when more information is available in this field the antibody- phenotype associations may change. While immunoprecipitation is more specific and sensitive it is not available for routine use. Therefore, understanding the results of line immunoblot in different populations and identifying appropriate titre cut-offs becomes important. A Dutch study,[4] analysed sera of IIM and non-IIM patients using cut-offs identical to our cut-off of ≥25 to define positivity for all autoantibodies. In this study, 18% of even non-IIM sera were MSA positive while 19% of IIM and 12.5% of non-IIM sera had multiple antibody positivity. suggests the need for critical awareness of the sensitivity of the line immunoblot assay.[8]

The EuroMyositis registry reported radiolabelled-immunoprecipitation for 23 antibodies in 1673 sera.[7] In this report, of the 62% sera in which MSAs/MAAs were detected, 85% had a single MSA while MAA was detected in 11%. The only previous report from India is a single centre cohort which evaluated SRP, Mi-2, Jo-1, PL-7, PL-12, EJ, OJ, Ro-52, Ku, PM-Scl75 and PM-Scl100 using similar immunoblot platform with results being reported semi-quantitatively and a ++ intensity being considered positive[8]. From the Indian subcontinent, through our study and the previous Indian study also we report a prevalence of multiple antibodies in almost 30% of patients. In our cohort the antibodies present in isolation were anti-TIF-1γ, SAE1 and EJ.

The 2017 ACR/EULAR criteria has been reported to be less sensitive than the Bohan-Peter criteria.[11,12] In our cohort of 16 patients who did not meet the score for classifying as IIM, 8 had antibodies present in sera, suggesting that adding more antibodies to the laboratory criteria of the ACR/EULAR score will improve the sensitivity of the criteria. Additionally, in the one third of patients, in whom the muscle biopsy variables listed in ACR/EULAR IIM classification criteria were available, it is clear that perifascicular atrophy is often found in patients with clinical features and autoantibody profiles consistent with OM or PM and not restricted to DM.

In our cohort while ANA was positive in 77.2% which is higher than all cohorts in which it is reported, there was no specific correlation between the presence of an MSA/ MAA antibody with either presence or pattern of ANA, though a speckled pattern was the commonest pattern though this as has been suggested previously.[13] ANA was positive in half of those who are MSA/MAA negative suggesting the possibility of discovering more antibodies/ those not covered by the 16 Ag myositis profile.

Strengths

This study derives from a multicentric cohort thus deriving from a population which is not restricted to one geographical area of the country.

Limitation

Since this report includes both prevalent and incident patients ongoing immunosuppressive therapy may have modified the titre of antibodies. The need to capture perspectives of experienced physicians in categorising subsets clinically and then looking at whether the antibodies added value resulted in a decision not to validate the clinician diagnosis by an independent observer. Evaluation for ILD/ cardiac disease was need based rather than uniform assessment methods across the entire cohort. A comparator non-IIM cohort or normal population was not included.

Conclusions

Almost one third of our entire cohort is autoantibody negative thereby suggesting yet unidentified antibodies involved in IIM initiation and perpetuation. MSA are often present in association with MAA. In Indian patients with IIM, ILD is associated with anti-synthetase and anti-Ro-52 antibodies. Cutaneous ulcerations are almost always associated with the presence of MSA/MAA in serum and specifically with anti-MDA5 and anti-PM-Scl antibodies. Dysphagia is associated with anti-Mi-2α, anti-Jo-1 and anti-TIF-1γ antibodies. Calcinosis was associated with anti-PM-Scl antibodies.

The best way forward to truly understand the relative importance of clinical features, autoantibodies and biopsy in clustering subsets of IIM to define treatment and prognosis is to have a cohort with all three accurately recorded and patient followed up longitudinally.

Acknowledgements

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