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ORIGINAL COMMUNICATION

Evaluating the diagnostic utility of new line immunoassays for myositis antibodies in clinical practice: a retrospective study

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Abstract

Background Myositis-associated antibodies (MAA) and myositis-specific antibodies (MSA) are detected in patients with idiopathic inflammatory myopathies (IIM); their role as diagnostic biomarkers is however still debated. The aim of our study was to assess the utility of MAA/MSA assessed by new line immunoassays in detecting myositis among neuromuscular patients.

Methods We retrospectively analysed sera samples obtained from patients tested for myositis antibodies with the "Euroline: Autoimmune Inflammatory Myopathies 16Ag" and "myositis profile 3" kits (Mi-2, TIF1γ, MDA5, NXP2, SAE1, Jo-1, SRP, PL-7/12, EJ, OJ, Ro-52, Ku, PM-Scl75/100). First symptom, CK, EMG, muscle biopsy and diagnosis were also analysed. Using logistic regression analysis, two diagnostic models were built to evaluate the diagnostic power of MAA/MSA in distinguishing myositis patients from controls and other myopathies.

Results 1229 patients were identified. 141 patients had a bioptic confirmed IIM; other diagnoses included: myopathy ($n=357$), other neuromuscular diseases ($n=144$) and no neuromuscular diseases ($n=587$). The specificity was 95% for MSA and 89% for MAA, the sensitivity 20% and 22%, respectively. MAA showed no use in differentiating myositis patients from controls, whereas MSA had limited effect (OR = 5.165), compared to other variables as EMG (OR = 47.755) or CK > 2000 U/L (OR = 45.307). MSA were, however, the most useful parameter differentiating IIM from non-IIM patients (OR = 7.259), better than CK > 2000 U/L (OR = 4.033) and MAA (OR = 2.737).

Conclusions Line immunoassays for myositis antibodies show high specificity but low sensitivity. Their usefulness as diagnostic biomarkers widely depends on the clinical settings. Our study suggests that MSA/MAA should be used for confirmatory and differential diagnosis rather than for screening purposes in inflammatory myopathies.

Keywords Idiopathic inflammatory myopathies · Myositis · Antibodies · Diagnosis

Introduction

Idiopathic inflammatory myopathies (IIM), also known as myositis, comprehend a group of rare diseases characterized by inflammation of skeletal muscle and variable involvement of the skin, the joints and the lungs. Five main entities are currently known: dermatomyositis (DM), immune-mediated necrotizing myopathy (IMNM), overlap myositis (OM), sporadic inclusion body myositis (sIBM) and the debated polymyositis (PM). This latter entity is in fact nowadays considered a diagnosis of exclusion of other IIM forms; many patients previously diagnosed as polymyositis may develop new clinical features suggestive of other diagnoses. For this reason, some authors even doubt the existence of PM as a distinct disease entity [1, 2].

In the last decade, an increasing number of myositis-specific (MSA) and myositis-associated antibodies (MAA) have been discovered in sera of patients with IIM. These autoantibodies seem helpful in classifying patients into the different IIM subtypes and predicting the disease course and outcome [3]. MSA display a high specificity but a relatively low prevalence of about 40–60% [4–6]. They are mostly mutually exclusive and include: Mi-2, NXP2,

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TIF-1γ, MDA5, SAE (usually associated with DM); SRP and HMGCoAR (associated with IMNM); Anti-Jo1, PL7, PL12, EJ, OJ, KS, Anti-Zo (associated with OM) [6]. The MAA are considered not specific for myositis, since they can be found also in other connective tissue diseases (e.g., systemic lupus erythematosus, Sjögren's syndrome, systemic sclerosis) and include, among others: anti-NT5C1a, found in a portion of sIBM patients, anti-Ku, anti-PM/Scl, anti-U1-RNP and anti-Ro-52 [7]. Some of these autoantibodies have a clear pathogenic role (SRP, HMGCoAR) [8], whereas others are considered to be an epiphenomenon, but still useful in the differential diagnosis of the IIM spectrum.

Several methods with variable sensitivity and specificity are used to detect MSA and MAA. The majority of studies on MSA detection and correlation with clinical features have been conducted adopting immunoprecipitation (IP), which is considered as the gold standard for detection of most autoantibodies. This technique is, however expensive, time consuming, subjective to interpretive errors and often implies the use of radioactive reagents. To overcome these limitations and to allow simultaneous detection of multiple autoantibodies, commercial multiplex dot or line immunoblots (LB) have been developed. These assays are nowadays increasingly adopted in the clinical setting; they are however not fully standardized and some still lack adequate validation. Comparing the performance of IP and LB, a concordance rate of only 77% was found with major differences regarding some autoantibodies; in particular, the higher discordance rate was found for anti-Jo-1 where LB showed a much lower sensitivity in comparison to IP [4]. Furthermore, the performances of different commercial LB kits vary among manufacturers (Alphadia, Euroimmun, Trinity Biotech) and some differences in specificity and sensitivity have been demonstrated between individual autoantibodies [9].

Even if a growing amount of evidence suggests that the presence of MSA and MAA plays an important role in diagnosis, prognosis and treatment of IIMs, their use in clinical practice is still limited in many countries and particularly in non-research settings. The most recently published diagnostic and classification criteria for IIM of the European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) do not include MSA/MAA (with the exception of anti-Jo-1) due to the lack of data in the studied population [10]. The steering committee of the Euromyositis Registry encourages therefore a systematic and harmonized collection of autoantibody data. Adopting validated assays and including comparator cases of non-IIM cases where MSA have been tested, is needed to allow future revision of the classification criteria possibly including MSA [11–13].

The aim of our study was therefore to determine the specificity and sensitivity of MSA and MAA tested with new line immunoassays in a large cohort of patients and to determine their diagnostic utility in the setting of a specialized neuromuscular outpatient clinic.

Patients and methods

In this study, we have included all patients that between 2014 and 2017 have been tested for the presence of MSA and MAA antibodies as part of the diagnostic process at our neuromuscular centre. The presence of myositis antibodies was assessed using either the immunoblot "myositis Profile 3" or the "autoimmune inflammatory myopathies 16 Ag" by "EUROIMMUN AG". These assays use membrane strips with antigens to detect the presence of myositis-related antibodies in patients´ sera. The "myositis Profile 3" detects the following antibodies: Mi-2, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ, Ro-52 separately. The "autoimmune inflammatory myopathies 16 Ag", in addition to all the above, also includes Mi-2 alpha/beta, TIF1γ, MDA5, NXP2 and SAE1. The analysis of the results was performed, following manufacturer´s recommendations, semi-quantitatively based on the signal intensity of each antibody. HGMCoAR antibodies and NT5c1A antibody have not been systematically tested in this cohort of patients, since they are not part of the above-mentioned assessment assays.

We have then retrospectively collected the following clinical features for each patient: diagnosis, symptom at onset, CK, EMG and muscle biopsy.

EMG findings have been classified into: (a) neurogenic (large, high-amplitude motor unit potentials—MUPs, poor interference pattern, with or without pathological spontaneous activity—psa); (b) myopathic (short, small MUPs, early/rich interference pattern with or without psa); (c) mixed pattern (a combination of the above with or without psa); (d) psa only (if only some psa was detected but MUPs characteristics, recruitment and interference patterns were interpreted as normal); and (e) normal EMG (none of the above).

All patients have been diagnosed and followed in our centre. Peter and Bohan criteria are adopted for the diagnosis of IIM. In addition, sIBM was diagnosed if suggestive clinical manifestations, and on muscle biopsy sections rimmed vacuoles, invasion of non-necrotic fibres, focal MHC1 expression were detected. Furthermore, in sIBM on semithin sections presence of myelin figures in rimmed vacuoles was seen. In selected cases, a filament visualization on electron microscopic investigation was done. IMNM was diagnosed if weakness and CK elevation were associated with necrotic fibres and no or only sparse inflammatory infiltrates in the muscle biopsy. In this latter case, the differential diagnosis with non-inflammatory myopathies depends on the disease course and response to therapy. The term overlap myositis includes anti-synthetase syndrome. We

have classified patients into four groups: (1) patients with a confirmed diagnosis of IIM; (2) patients with a genetically or histologically confirmed non-inflammatory myopathy; (3) patients with other neuromuscular diseases (e.g., ALS, neuropathies, myasthenia); (4) patients without overt neuromuscular diseases.

Ethics statement: The study was fully performed under the Helsinki Declaration. Due to the retrospective nature of our study, we did not analyse the outcomes of interventions in patient cohort. As we did not provide identifiable patient data, this study was without a formal need of approval by our local ethics committee.

Statistical analysis

All analyses were performed using the statistical software R 3.3.2. Descriptive statistics has been performed where applicable. We estimated the overall sensitivity (Se) and specificity (Sp) of MSA (Mi-2, SRP, Jo-1, EJ, OJ, Pl-7, Pl-12, SAE-1, Tif-1γ, Nxp-2, Mda-5) and MAA (Ro-52, PM-Scl75, PM-Scl100, Ku), respectively, as well as for each antibody. We considered the test positive if any antibody of the MSA (and then MAA) panel was positive. For each estimate, we calculated a 95% confidence interval (CI), and positive (LR+) and negative (LR−) likelihood ratios for MSA and MAA.

To assess the diagnostic value of MAA/MSA in the clinical setting, we used multivariable logistic regression to build two diagnostic models: (1) differentiating IIM patients from those who did not have a neuromuscular disease; and (2) differentiating patients with IIM from those having a non-inflammatory myopathy. We used the following variables for building the models: MSA positive, MAA positive, CK levels (numerical and categorical), age (numerical and categorical), gender, first symptom reported (fatigue, myalgia, muscle weakness, elevated CK, or other), and EMG findings (neuropathic, myopathic, mixed pattern, pathological spontaneous activity only or normal). We used the backward stepwise approach to build the models. We fitted the models based on the Akaike information criteria (AIC) and the amount of variance explained by the model (assessed with McFadden's pseudo-R^2). By running the models on the same dataset, we studied the receiver operating characteristic (ROC) with the area under the curve (AUC) as an indicator of model performance and accuracy. To adjust for optimism, we used bootstrapping to recalculate the AUC ($AUC_{BOOTSTRAP}$).

Sensitivity analyses of the study

To address potential bias, we performed additional analyses. Some patients were assessed only with the "myositis Profile 3"; we stratified the analysis for these patients (49 myositis patients and 389 controls) to test the potential confounding due to using different assays. Since patients with sIBM do not usually exhibit MSA/MAA antibodies, we repeated the entire main analysis excluding patients with sIBM ($n = 29$), to see whether this subsample has affected the Se and Sp estimates. In addition, we have repeated the entire analysis using only the observations from those patients who had a muscle biopsy, to test the uncertainty of the diagnosis, by including only those patients with a bioptic diagnosis of IIM.

Results

Between 2014 and 2017, 1727 serum samples (1535 patients) were tested for MSA and MAA in our centre. Clinical data were available for 1229 patients (1414 serum samples) which were included in the study. A muscle biopsy was performed in 582 patients (47%). 535 sera (441 patients) were tested with the kit "autoimmune inflammatory myopathies 16 Ag" (38%) and the remaining 879 sera (788 patients) with the "myositis profile 3".

141 patients had a finally confirmed diagnosis of IIM (11.5%); 357 patients a non-inflammatory myopathy (32.8%); 144 patients other neuromuscular diseases (13.2%); and in 587 patients a neuromuscular disease was ruled out (53.9%). Within the IIM group of 141 patients, the following diagnoses were reported: immune-mediated necrotising myopathy (IMNM) in 38 patients (26.95%), sporadic inclusion body myositis (sIBM) in 29 patients (20.56%), polymyositis or unspecific myositis (PM) in 26 patients (18.43%), dermatomyositis (DM) in 24 patients (17.02%), overlap myositis (OM) in 24 patients (17.02%) (Fig. 1).

No statistically significant differences were present as regards age and gender distribution between IIM patients and non-IIM patients. Of 141 IIM patients, 67 were women (47.52%), and the mean age at diagnosis was 59.47 years (SD 14.27). Among 1088 non-IIM controls, 476 were women (43.75%). Mean age of the non-IIM sample was 51.47 years old (SD 15.83).

A positive result for either MSA or MAA was found in 36% of IIM patients. The concomitant occurrence of different MSA was found in only 11 out of 1414 sera, of whom 7 patients, 3 with a diagnosis of IIM, corresponded to 0.78% of assays (2.63% of IIM patients). The co-occurrence of MSA and MAA was detected in 30 out of 1414 sera (2.12%), of those 11 out of 141 IIM patients (7.8%). Among 1229 patients, 140 had a repeated myositis panel at least twice. The reproducibility of the repeated measurements was high (Pearson's $r = 0.76$).

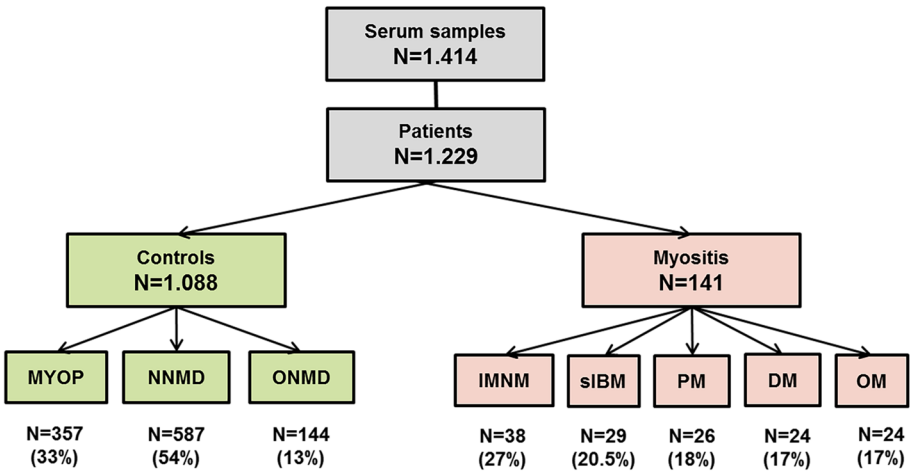

Fig. 1 Description of the study cohort

Sensitivity and specificity

The MSA showed a very high specificity of 95.13% (95% CI 93.85–96.41%) but a low sensitivity of 19.86% (95% CI 13.27–26.44%). Similarly, MAA had a lower specificity of 89% and a slightly higher sensitivity (22%) (Table 1).

MSA had a positive likelihood ratio (LR+) of 4.08 (95% CI 2.67–6.22), whereas for MAA it was 2.04 (95% CI 1.43–2.92). The negative likelihood ratio (LR−) was 0.84 (95% CI 0.78–0.92) and 0.87 (95% CI 0.80–0.96), for MSA and MAA, respectively. The results of Se and Sp for MSA and MAA as well for each antibody are shown in Fig. 2.

Diagnostic value of the antibodies

To estimate the role of MSA and MAA antibodies in the setting of a neuromuscular outpatient clinic, we build multivariable logistic regression models to identify which predictors are important in differentiating IIM patients from patients without a neuromuscular disease (healthy controls—model 1) and from patients with a non-inflammatory myopathy (model 2). The size of the diagnostic value of each statistically significant variable is represented with the odds ratio (OR).

In model 1, we have compared the 141 IIM patients versus the 587 patients without an overt neuromuscular disease (Table 2).

In this scenario, the following variables were statistically significant predictors of myositis as an outcome: age (numerical, in years), CK (as categories), presence of muscle weakness at onset, myopathic EMG and any positive MSA antibody. The ORs show that the most important positive predictor for a diagnosis of myositis is a myopathic EMG (OR = 47.76), followed by CK levels above 2,000 U/L (OR = 45.30), and muscle weakness (OR = 37.07). In this setting, positive MSA antibodies are still statistically significant predictors having, however, a lower diagnostic power (OR = 5.17) in differentiating myositis from controls compared to the other predictors (Table 2). This model shows excellent accuracy (AUC = 0.946) in differentiating myositis patients from controls (Fig. 3a).

In model 2, we have compared the 141 IIM patients versus the 357 patients with a non-inflammatory myopathy (Table 3).

In this second scenario, the following variables were statistically significant predictors of myositis as an outcome: age (numerical, in years), any positive MSA, any positive MAA, myalgia at onset, normal EMG, and CK

Table 1 Sensitivity and specificity analysis

	MSA		MSA		MAA		MAA	
	Sp	95% CI	Se	95% CI	Sp	95% CI	Se	95% CI
Overall ($n = 1229$)	95.12	93.84–96.40	19.85	13.27–26.44	89.24	86.37–92.12	21.98	15.14–28.82
Exclusion of sIBM ($n = 1200$)	95.12	93.84–96.40	24.34	16.50-32.19	89.24	86.37–92.12	21.73	14.20-29.27
16Ag Kit only ($n = 441$)	92.80	90.23–95.37	24.48	12.44–36.53	87.66	84.39–90.92	14.28	4.48–24.08
MuBi Pts only ($n = 582$)	94.27	92.13–96.41	19.37	12.55–26.20	88.76	85.86–91.67	21.70	14.59–28.81

Additional data on sensitivity analysis as supplementary material

MSA myositis-specific antibodies, *MAA* myositis-associated antibodies, *Sp* specificity, *Se* sensitivity, *sIBM* sporadic inclusion body myositis, *MuBi* muscle biopsy, *Pts* patients

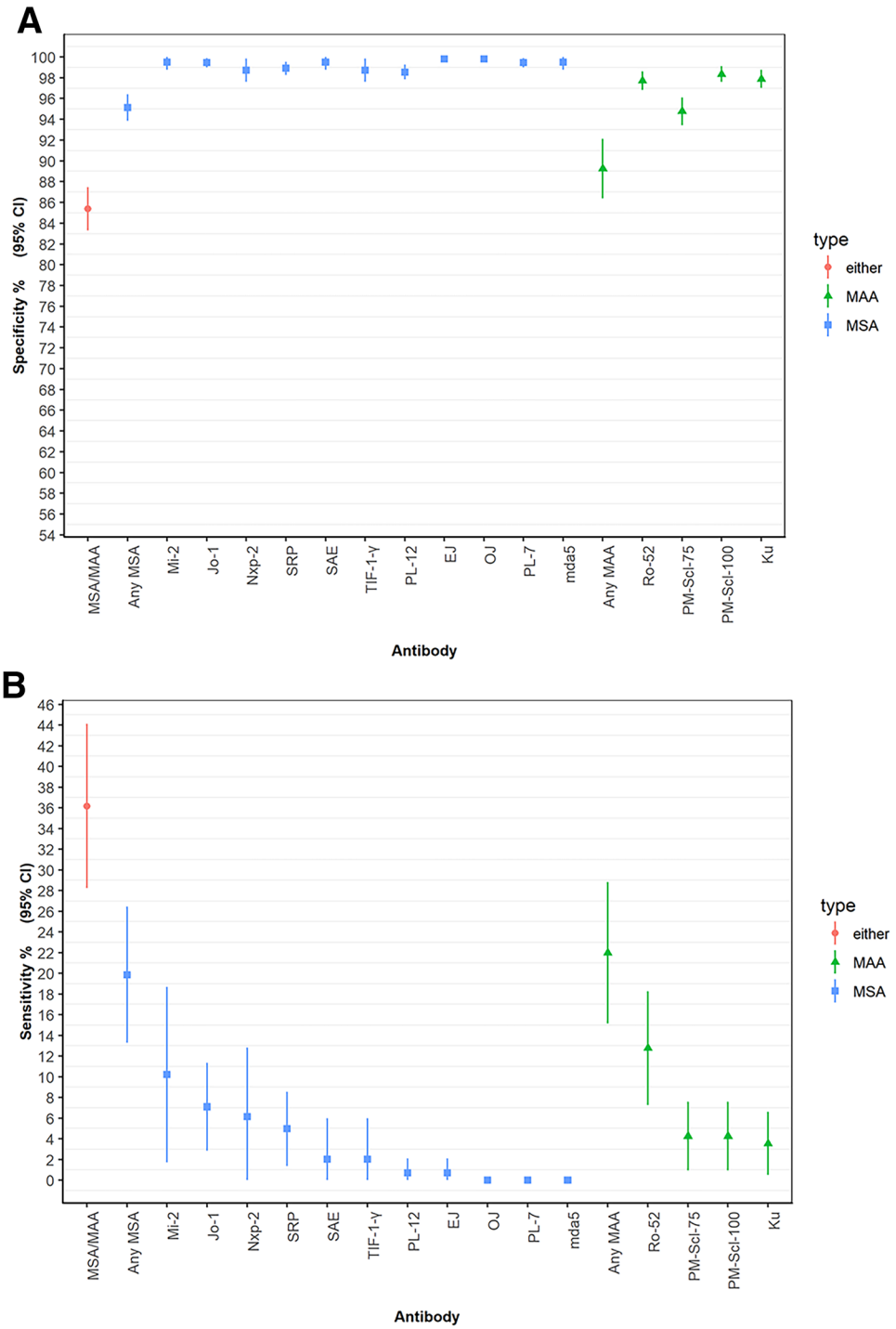

Fig. 2 **a** Specificity of MSA, MAA, and each antibody separately; **b** sensitivity of MSA, MAA, and each antibody separately. The shape points represent the estimates of Se/Sp, whereas the lines represent the 95% confidence intervals

(as categorical). All of them increase the odds of having myositis, except for normal EMG, which decreases the odds of having myositis. In this setting, the MSA antibodies had the highest diagnostic value (OR = 7.25), as compared to the other variables (Table 3). This model shows a good accuracy (AUC = 0.796) in differentiating myositis patients from those with non-inflammatory myopathies (Fig. 3b).

Figure 4 represents a heat map of the prevalence of antibody positivity found in each IIM subtype (Fig. 4).

Sensitivity analyses of the study

No meaningful alteration of the findings was detected. In Table 1, we report the Se and Sp estimates calculated in the sensitivity analyses, after excluding the sIBM patients, after

Table 2 Model 1: diagnostic model of differentiating IIM patients from patients with no neuromuscular disease

Variable	Log estimate	Standard error	*p* value	Odds ratio (OR)	OR 95% CI
Intercept	− 5.205	0.754	5^{-12}***	0.005	0.001–0.024
Age (in years)	0.026	0.013	3^{-02}*	1.027	1.002–1.052
CK 350–1000 U/L	1.394	0.361	1^{-04}***	4.033	1.987–8.186
CK 1000–2000 U/L	3.237	0.889	3^{-04}***	25.451	4.457–145.345
CK > 2000 U/L	3.813	1.069	4^{-04}***	45.307	5.576–368.154
Weakness at onset	3.613	0.466	9^{-15}***	37.069	14.872–92.398
Myopathic EMG	3.866	0.561	5^{-12}***	47.755	15.916–143.287
Any MSA+	*1.642*	*0.457*	*3^{-04}****	*5.165*	*2.111–12.637*

Italics to highlight the performance of MSA. AIC: 276.62, null deviance: 715.65, residual deviance: 260.62, Mc Fadden's R^2: 0.636

CK creatine kinase, *EMG* electromyography, *MSA* myositis-specific antibodies

*p value < 0.05, **p value < 0.01, ***p value < 0.001

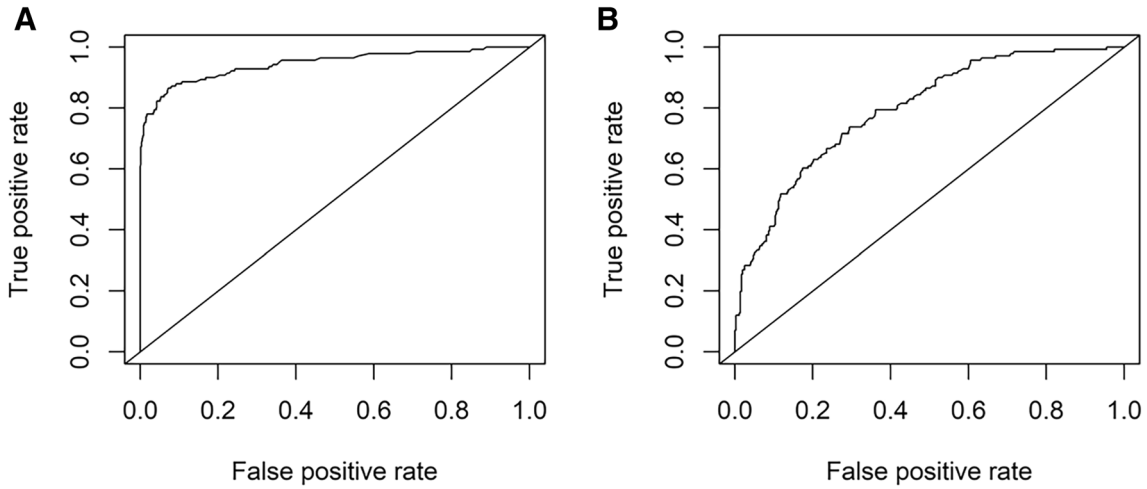

Fig. 3 Receiver operating characteristic (ROC) with the area under the curve (AUC) as an indicator of model performance and accuracy. **a** Model 1 on the left AUC = 0.946 (95% CI 0.920–0.971). **b** Model 2 on the right AUC = 0.796 (95% CI 0.754–0.838)

Table 3 Model 2: diagnostic model differentiating IIM from non-inflammatory myopathy patients

	Estimate	Standard error	Stat. significance (*p*)	*p* value	Odds ratio (OR)	OR 95% CI
Intercept	− 4.116	0.542	3E−14	***	0.016	0.006–0.047
Age (in years)	0.043	0.008	1E−07	***	1.044	1.027–1.060
Any MSA+	*1.982*	*0.420*	*2E−06*	***	*7.259*	*3.188–16.530*
Any MAA+	*1.007*	*0.315*	*1E−03*	**	*2.737*	*1.475–5.079*
Myalgia at onset	1.451	0.321	6E−06	***	4.269	2.277–8.003
Normal EMG	− 1.065	0.315	7E−04	***	0.345	0.186–0.639
CK 500–1000 U/L	0.822	0.281	3E−03	**	2.276	1.311–3.950
CK 1000–2000 U/L	1.144	0.376	2E−03	**	3.138	1.501–6.563
CK > 2000 U/L	1.394	0.411	7E−04	***	4.033	1.802–9.023

Italics to highlight the performance of MSA/MAA. AIC: 486.67, null deviance: 593.5, residual deviance: 468.67, Mc Fadden's R^2: 0.210

CK creatine kinase, *EMG* electromyography, *MAA* myositis-associated antibodies, *MSA* myositis-specific antibodies

*p value < 0.05, **p value < 0.01, ***p value < 0.001

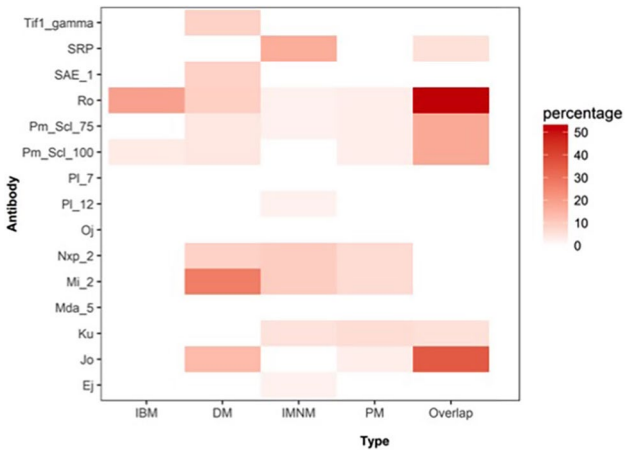

Fig. 4 Prevalence of MSA and MAA antibodies in the different IIM subtypes. The colour of the boxes indicates the % of positivity among IIM patients. *IMNM* immune-mediated necrotizing myopathy, *DM* dermatomyositis, *IBM* inclusion body myositis, *PM* polymyositis (also including unspecific myositis), *Overlap* overlap myositis

considering only patients tested with the extensive "autoimmune inflammatory myopathies 16 Ag" kit or after repeating the analysis only in those who had a biopsy-confirmed diagnosis. The findings of the regression analyses showed only slight changes in the OR and AUC estimates, which is expected due to the change in sample sizes; the OR estimates remained in the same direction as in the main analysis (see supplementary material).

Discussion

In time, many diagnostic criteria for IIMs have been proposed, starting from the well-known Bohan and Peter criteria of 1975 and arriving to the most recent diagnostic and classification criteria proposed by the EULAR/ACR [10, 14, 15]. The combination of clinical, serological and histological features usually allows the classification of patients into one of the above-mentioned IIM types. Nevertheless, some overlap among these entities exists complicating both diagnosis and management of patients. Muscle biopsy is still considered as the most powerful diagnostic tool for confirming the IIM diagnosis and classifying patients into the different IIM subtypes. However, its diagnostic accuracy is investigator experience dependent, and may vary depending on how severely affected is the biopsied muscle; some pathognomonic features, such as rimmed vacuoles in sIBM, may be absent in early stages of the disease, some patients may mainly complain of extramuscular manifestations of IIM without overt muscle damage.

Thanks to the discovery of MSA/MAA and to an improved knowledge of distinct clinical features of IIM subtypes, the key diagnostic role of muscle biopsy for IIMs is now relined by clinical and serological bystanders, so that, for example, it is not considered anymore needed in classical DM patients [10].

Some authors greatly emphasize the diagnostic utility of MSA for IIM classification, suggesting that morphological analysis may be dispensable if MSA are found in patients with the corresponding phenotype [16]. However, a high degree of uncertainty still exists as regards the diagnostic performance of MSA/MAA in clinical practice. The low prevalence of MSA in IIM patients (usually 30–50%) [6, 17, 18], the discrepancies among different assessment methods and among manufacturers [4, 9], the lack of data on non-myositis patients are the major determinants for such uncertainty. This determined the exclusion of MSA/MAA assessment from the EULAR/ACR diagnostic criteria [10], raising some debate in the field [12]. In an attempt to overcome some of these uncertainties, our study aimed at analysing the diagnostic performance of immunoblot assays to assess its utility in clinical practice.

We analysed the results of new line immunoassays for MAA and MSA performed on a large retrospective cohort of myositis and non-myositis patients in our neuromuscular centre. Having included in the analysis a high number of controls contributes to more valid estimates of sensitivity and specificity in clinical practice. Similar studies have been recently performed by other authors on a far lower number of IIM patients, adopting other immunoassays or not including a control group [6, 17–20].

Overall, the performance of the investigated EUROLINE kits was good in regard to its high specificity of 95% and 89% found for MSA and MAA, respectively. On the other hand, the sensitivity of 20–22% for MSA and MAA was lower in comparison to what reported by other studies [5, 6, 9]. Ghirardello et al. reports a prevalence of antigen-specific antibodies (MAA or MSA) in about 60% of myositis patients; another recent study also adopting the Euroline 16 Ag kit, found a prevalence of 77% of MSA/MAA in IIM patients diagnosed according to the most recent EULAR/ACR diagnostic criteria [6]. To explain the low prevalence of MSA/MAA in our study (36%), the following considerations can be made. In our IIM cohort, the larger group of patients had an IMNM which is usually associated with anti-SRP or anti-HMGCoAR antibodies (Ab), the latter are not included in the EUROLINE kit thus contributing to the low prevalence. The IIM diagnosis in our study was recorded retrospectively from analysed medical records adopting Peter and Bohan criteria. These criteria are nowadays increasingly replaced by the most recently developed EULAR/ACR criteria, which might have helped selecting a better-defined myositis cohort. Also, many patients have been diagnosed as "polymyositis" or "unspecific myositis", terms that today are decreasingly used and might masquerade other diagnoses (as

sIBM without rimmed vacuoles or IMNM) [2, 16]. Even the different disease activity among patients might have contributed to the low MSA/MAA prevalence, as the levels of some MSA are known to fluctuate during the disease course [21, 22]. The use of two different immunoassays and the inclusion of sIBM patients in our analysis cannot be accounted as an explanation for the low sensitivity, since no meaningful alteration of findings was detected with the sensitivity analyses (Table 1 and supplementary material).

Despite their low sensitivity, MSA were still found to be statistically significant predictors of myositis as an outcome in our study in both explored clinical scenarios (model 1 and model 2). MAA, on the contrary, only played a role in distinguishing between myositis and hereditary myopathy patients.

These results support the importance of including not only MSA but also MAA in future diagnostic criteria. The relevance of MSA/MAA positivity in the diagnostic process should be however interpreted depending on the clinical context. Model 1 shows that to exclude the presence of an IIM, other diagnostic tools (clinical examination, EMG, CK) have a higher impact in predicting the presence of myositis in comparison to MSA. This, together with their low sensitivity, suggests that MSA/MAA assays should not be used as screening tools. In a context of high clinical suspicion for IIM instead, as in model 2, MSA and MAA were the most powerful discriminants and predictors for myositis as an outcome, thus highlighting their utility as confirmatory test. These results are in line with previous studies showing that patients with hereditary myopathies, even with signs of inflammation in muscle biopsy as in facioscapulohumeral dystrophy, do not usually display MSA antibodies [23, 24]. The low prevalence of MSA should be kept in mind not only in the diagnosis but also in the follow up of patients; since some TIFγ positive patients may be missed, an accurate, guideline-driven, cancer screening should be considered also in seronegative IIMs patients especially in those with dermatomyositis.

In this study, we would also like to emphasize the key role of EMG in the diagnostic process of IIM. This tool has been neglected in the EULAR/ACR diagnostic criteria due to the low number of myositis patients who received an EMG, especially in rheumatological centre settings [10]. In both examined scenarios, EMG was a relevant predictor for myositis, especially considering the negative predictive value of a normal EMG (Table 3). These findings are in line with two recent and almost simultaneously published papers comparing EMG and histological findings, highlighting the high negative predictive value (82–93%) of the absence of fibrillation potentials for inflammation [25, 26].

Another critical issue in the interpretation of MSA/MAA regards the clinical–serological correlations. As shown in the heatmap represented in Fig. 4, most of the clinical associations between each MSA/MAA and different IIM subtypes were confirmatory of what already reported in the literature.

TIF1γ, SAE1, NXP2 and Mi-2 antibodies were predominantly detected in DM patients, and anti-SRP antibodies were mainly found in IMNM patients, and Jo-1 in OM. MAA did not show any specific association with an IIM subtype. Yet they were slightly more prevalent in the OM group of patients.

Some clinical–serological discrepancies were however found in this and in other similar studies [6, 19]. Jo-1 antibodies were, for example, found also in DM patients, SRP in OM, NXP2 and Mi2 in IMNM. This is mainly related to the known difficulties in differentiating these forms clinically and histologically:[27] skin manifestations can be detected in both DM and anti-synthetase syndrome (ASS), the histological differentiation between OM, IMNM and DM may be troublesome as some DM patients may have only a low amount of inflammation, and some PM cases will probably be reclassified into other IIM subtype as symptoms evolve. In a neuromuscular clinical setting, great value is given to the results of muscle biopsy, so that in this retrospective study the diagnosis of IIM subtypes was mainly achieved considering the histological and clinical features. The introduction of MSA/MAA detection in clinical practice may then cause some discrepancies in those cases where the result of muscle biopsy did not clearly point towards a specific IIM subtype. According to the EULAR/ACR criteria, adult IIM patients can be subdivided into PM, IBM, DM or amyopathic DM, based on clinical findings and muscle biopsy features. Due to the low number of patients, the IMNM and OM subgroups could not be defined in the EULAR study [10]. Thereafter, Mariampillai et al. demonstrated that MSA were the most relevant variable estimating the belonging of patients to a specific IIM cluster. The clusters they found corresponded to DM, IBM, IMNM and ASS; no case of PM was found and no diagnostic criteria have been proposed [16].

In this study, we could prove the utility of the MSA/MAA as diagnostic biomarkers for IIM, however, some discrepancies emerged when considering the association between specific MSA/MAA and IIM subtypes. Such clinical–serological discrepancy occurred also in other studies that adopted the EULAR/ACR diagnostic criteria and then examined patients for MSA/MAA [6]. To solve this diagnostic uncertainty, there is a compelling need for integrating all relevant diagnostic tools for IIM (clinical features, CK, MAA/MSA, EMG, muscle biopsy and possibly muscle MRI) in more comprehensive and updated diagnostic and classification criteria. The ongoing prospective data collection in the EuroMyositis registry will hopefully be the tool to achieve this goal.

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Compliance with ethical standards

Conflicts of interest All authors declare they have no conflict of interest to disclose.

Ethical standards The study has been conducted in compliance with the principles of the declaration of Helsinki and with the local German laws and regulations.

References

1. Selva-O'Callaghan A, Pinal-Fernandez I, Trallero-Araguas E et al (2018) Classification and management of adult inflammatory myopathies. Lancet Neurol 17(9):816–828
2. Amato AA, Griggs RC (2003) Unicorns, dragons, polymyositis, and other mythological beasts. Neurology 61(3):288–289
3. Betteridge Z, McHugh N (2016) Myositis-specific autoantibodies: an important tool to support diagnosis of myositis. J Intern Med 280(1):8–23
4. Cavazzana I, Fredi M, Ceribelli A et al (2016) Testing for myositis specific autoantibodies: Comparison between line blot and immunoprecipitation assays in 57 myositis sera. J Immunol Methods 433:1–5
5. Ghirardello A, Zampieri S, Tarricone E et al (2006) Clinical implications of autoantibody screening in patients with autoimmune myositis. Autoimmunity 39(3):217–221
6. Zampeli E, Venetsanopoulou A, Argyropoulou OD et al (2018) Myositis autoantibody profiles and their clinical associations in Greek patients with inflammatory myopathies. Clin Rheumatol. https://doi.org/10.1007/s10067-018-4267-z
7. Palterer B, Vitiello G, Carraresi A et al (2018) Bench to bedside review of myositis autoantibodies. Clin Mol Allergy 16:5. https://doi.org/10.1186/s12948-018-0084-9
8. Ladislau L, Arouche-Delaperche L, Allenbach Y et al (2018) Potential pathogenic role of anti-signal recognition protein and anti-3-hydroxy-3-methylglutaryl-CoA reductase antibodies in immune-mediated necrotizing myopathies. Curr Rheumatol Rep 20(9):56. https://doi.org/10.1007/s11926-018-0763-z
9. Vulsteke JB, De Langhe E, Claeys KG et al (2018) Detection of myositis-specific antibodies. Ann Rheum Dis. https://doi.org/10.1136/annrheumdis-2017-212915
10. Lundberg IE, Tjarnlund A, Bottai M et al (2017) 2017 European League Against Rheumatism/American College of rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. Arthritis Rheumatol (Hoboken, NJ) 69(12):2271–2282
11. Tjärnlund A, Rönnelid J, Bottai M et al (2018) Response to: 'Detection of myositis-specific antibodies' by Vulsteke et al. Ann Rheum Dis. https://doi.org/10.1136/annrheumdis-2018-212948
12. Mahler M, Fritzler MJ (2018) Detection of myositis-specific antibodies: additional notes. Ann Rheum Dis. https://doi.org/10.1136/annrheumdis-2018-213153
13. Tjärnlund A, Lundberg IE, Rönnelid J (2018) Response to: 'Detection of myositis-specific antibodies: additional notes' by Infantino et al. Ann Rheum Dis. https://doi.org/10.1136/annrheumdis-2018-213341
14. Bohan A, Peter JB (1975) Polymyositis and dermatomyositis (second of two parts). N Engl J Med 292(8):403–407
15. Bohan A, Peter JB (1975) Polymyositis and dermatomyositis (first of two parts). N Engl J Med 292(7):344–347
16. Mariampillai K, Granger B, Amelin D et al (2018) Development of a new classification system for idiopathic inflammatory myopathies based on clinical manifestations and myositis-specific autoantibodies. JAMA Neurol. https://doi.org/10.1001/jamaneurol.2018.2598
17. Lecouffe-Desprets M, Hémont C, Néel A et al (2018) Clinical contribution of myositis-related antibodies detected by immunoblot to idiopathic inflammatory myositis: a one-year retrospective study. Autoimmunity 51(2):89–95. https://doi.org/10.1080/08916934.2018.1441830
18. Dobloug C, Garen T, Bitter H et al (2015) Prevalence and clinical characteristics of adult polymyositis and dermatomyositis; data from a large and unselected Norwegian cohort. Ann Rheum Dis 74(8):1551–1556. https://doi.org/10.1136/annrheumdis-2013-205127
19. Gofrit SG, Yonath H, Lidar M et al (2018) The clinical phenotype of patients positive for antibodies to myositis and myositis-related disorders. Clin Rheumatol 37(5):1257–1263. https://doi.org/10.1007/s10067-018-4032-3
20. Cruellas MG, Viana Vdos S, Levy-Neto M et al (2013) Myositis-specific and myositis-associated autoantibody profiles and their clinical associations in a large series of patients with polymyositis and dermatomyositis. Clinics (Sao Paulo) 68(7):909–914. https://doi.org/10.6061/clinics/2013(07)04
21. Benveniste O, Drouot L, Jouen F et al (2011) Correlation of anti-signal recognition particle autoantibody levels with creatine kinase activity in patients with necrotizing myopathy. Arthritis Rheum 63(7):1961–1971. https://doi.org/10.1002/art.30344
22. Stone KB, Oddis CV, Fertig N et al (2007) Anti-Jo-1 antibody levels correlate with disease activity in idiopathic inflammatory myopathy. Arthritis Rheum 56(9):3125–3131
23. Hengstman GJ, van Brenk L, Vree Egberts WT et al (2005) High specificity of myositis specific autoantibodies for myositis compared with other neuromuscular disorders. J Neurol 252(5):534–537
24. Mammen AL, Casciola-Rosen L, Christopher-Stine L et al (2015) Myositis-specific autoantibodies are specific for myositis compared to genetic muscle disease. Neurol Neuroimmunol Neuroinflamm 2(6):e172. https://doi.org/10.1212/NXI.0000000000000172
25. Sener U, Martinez-Thompson J, Laughlin RS, Dimberg EL, Rubin DI (2018) Needle electromyography and histopathologic correlation in myopathies. Muscle Nerve. https://doi.org/10.1002/mus.26381
26. Naddaf E, Milone M, Mauermann ML, Mandrekar J, Litchy WJ (2018) Muscle biopsy and electromyography correlation. Front Neurol 9:839. https://doi.org/10.3389/fneur.2018.00839
27. Lundberg IE, de Visser M, Werth VP (2018) Classification of myositis. Nat Rev Rheumatol 14(5):269–278. https://doi.org/10.1038/nrrheum.2018.41