Autoantibodies to Mi-2 alpha and Mi-2 beta in patients with idiopathic inflammatory myopathy

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Abstract

Objectives. The objective of this study was to compare the results obtained from different assays for the detection of anti-Mi-2 antibodies, which are important markers in the diagnosis of DM.

Methods. The study included 82 patients (68 females/14 males), most of whom had DM (n = 57), followed by PM (n = 16) and juvenile DM (n = 9). All samples were tested using a novel particle-based multi-analyte technology (PMAT) (Inova Diagnostics, research use only) in parallel with a line immunoassay (LIA: Euroimmun). To assess clinical specificity for the PMAT assay, a total of 775 disease and healthy controls were tested.

Results. 29 samples were positive by at least one test for anti-Mi-2 antibodies. Of those, 24 were Mi-2a LIA+, five were Mi-2a LIA+ and 23 Mi-2 PMAT+. The comparison shows varying agreement between the different methods (kappa 0.27/-0.77). When LIA results were used as reference for receiver operating characteristics analysis, high area under the curve values were found for both PMAT vs LIA Mi-2a and LIA Mi-2β. When analysing the results in the context of the myositis phenotype, PMAT associated closest with the DM phenotype. In the control group, 3/775 controls (all low levels) were anti-Mi-2+ resulting in a sensitivity and specificity of 28.1% and 99.6%, respectively.

Conclusion. Overall, good agreement was found between LIA and PMAT for anti-Mi-2 antibodies, which is important for the standardization of autoantibodies. Anti-Mi-2β antibodies measured by PMAT tend to be more highly associated with the clinical phenotype of DM.

Key words: autoantibodies, myositis, DM, anti-Mi-2 antibodies, immunoassay

Rheumatology key messages

- Isoform Mi-2β appears to be sufficient for the detection of anti-Mi-2 antibodies in myositis.
- Diagnostic platform has an impact on the accuracy of the results.
- Results derived from novel particle-based multi-analyte technology agree well with the DM phenotype and immunofluorescence pattern.
Introduction

Myositis-specific antibodies (MSA) and myositis-associated antibodies (MAA) have been used as an aid in the diagnosis of idiopathic inflammatory myopathies (IIM) [1]. Especially over the past 10–15 years, many novel and clinically relevant MSA have been identified that can help in the diagnosis and stratification of IIM [1]. Just recently, the newly published classification criteria for IIM [2, 3] triggered a debate about why MSA (except anti-Jo-1 antibodies) were not included, which was eventually explained by the lack of standardization of MSA assays and missing data derived from large published multicentre studies [4, 5].

Anti-Mi-2 antibodies were the first autoantibodies specific for DM recognized by double immunodiffusion using calf thymus extract as the antigen source [1, 6]. Mi-2 is a helicase of the nucleosome remodelling deacetylase multi-protein complex with histone deacetylase/demethylase activities. Anti-Mi-2 autoantibodies immunoprecipitate two proteins, Mi-2α and Mi-2β, of 220 and 218 kDa, respectively. Other nucleosome remodelling deacetylase complex proteins co-precipitate at 200, 150, 75, 65, 63, 50 and 34 kDa [7]. Indirect immunofluorescence (IIF) on HEP-2 cells reveals a fine, tiny speckled pattern in interphase nuclei and in metaphase cells chromatin is not stained but the dispersed nucleoplasm presents the same fine, tiny speckles. Commercial ELISA and immunoblot kits identify anti-Mi-2 autoantibodies [8, 9]. Anti-Mi-2 are commonly detected in DM patients, either in adults (11–59%) or in children (4–10%), with a great variability among the studies [8–11]. Their presence in PM and sporadic inclusion body myositis (sIBM) is less common [10]. The Mi-2 protein is over-regulated during muscle regeneration in DM patients and thought to be related to ultraviolet exposure, sex and HLA DRB1*0302 and DRB1*0701. Anti-Mi-2 positive DM patients usually exhibit a mild myopathy despite high creatine kinase levels, without lung involvement and/or cancer [1, 12]. Overall, anti-Mi-2 positivity is associated with a good prognosis and a favourable response to corticosteroids [13]. DM constitutes a significantly higher portion of IIM in patients from Mexico [14] and there is a reported increased prevalence of anti-Mi-2 antibodies in DM from other geographic regions [15]. Consequently, IIM cohorts from Mexico represent an ideal population to evaluate assays for the detection of anti-Mi-2 antibodies.

Certain reference laboratories use immunoprecipitation (IP) to detect MSA but widespread use of IP is fraught with logistic, standardization and regulatory challenges [16], especially in high throughput diagnostic laboratories. Therefore, reliable alternatives to IP are mandatory. The aim of the present study was to compare a commonly used line immunoassay (LIA) and a novel particle-based multi-analyte technology (PMAT) system for the detection of anti-Mi-2 antibodies. In addition, we aimed to study the utility of the novel PMAT system on a large cohort of patients and controls.

Methods

Patients

The study included 82 consecutive patients (68 females/14 males), most of whom had DM (n = 57), followed by PM (n = 16) and juvenile DM (n = 9). All samples were tested using a novel PMAT system (Inova Diagnostics, research use) in parallel with an LIA (Euroimmun GmbH, Luebeck, Germany, not Food and Drug Administration approved). An extended cohort of controls was used for the evaluation of the PMAT system including patients with systemic sclerosis (SSc, n = 472); mixed connective tissue disease (n = 21); SLE (n = 8); RA (n = 15); osteoarthritis (n = 49); Crohn’s disease (n = 15); infectious diseases (n = 47); primary Raynaud phenomenon (n = 13) and other conditions (n = 29). Patient data was anonymously used in keeping with the latest version of the Helsinki Declaration of human research ethics. Collection and processing of patient serum samples was carried out according to local ethics committee regulations (Research and Ethics Committee of Hospital General de Occidente). The study was carried out according to the DIRECTIVE 98/79/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL and the German law for medical device (Medizinproduktegesetz).

Line immunoassay

The LIA [EUROLINE Autoimmune Inflammatory Myopathies 15 Ag (DL 1530–1601-4 G); Euroimmun GmbH] used in this study is designed to detect antibodies to OJ, EJ, PL-12, PL-7, signal recognition particle (SRP), Jo-1, PM/SCi75, PM/SCi100, Ku, small ubiquitin-like modifier activating enzyme (SAE), nuclear matrix protein 2 (NXP2), melanoma differentiating antigen 5 (MDA5), transcriptional intermediary factor 1 (TIF1γ), Mi-2β and Mi-2α. After testing the samples, LIA strips were processed with a EUROBiotMaster (Euroimmun GmbH, YG 0151-0101) and reactivity (band intensity) digitized and quantitated by EUROLineScan (Euroimmun GmbH, YG 0006-0101) that includes a proprietary software system.

Particle-based multi-analyte technology (PMAT)

All samples were tested using PMAT (Inova Diagnostics, San Diego, CA, US; research use only), which included Mi-2β, OJ, TIF1γ, PL-7, PL-12, SAE, EJ, MDA5, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), SRP and NXP2. The principle of this novel diagnostic platform is based on paramagnetic particles coupled to antigens. Each antigen was coupled on a different type of particle, which are differentiated from each other based on a unique signature. In a first incubation step, the diluted serum samples were added to a mixture of the particles, pre-coupled with analyte-specific antigens. During incubation, specific antibodies (if present) bound to the cognate analytes (autoantigens). Unbound antibodies were removed by a washing step followed by incubation in a solution containing phycoerythrin-conjugated secondary antibody. After a second washing step, the particles were transferred to a chamber where two
spectrally distinct light-emitting diodes illuminated the particles. One light-emitting diode identified the particles (and consequently, the analyte that was detected) and the second light-emitting diode determined the magnitude of the phycoerythrin-derived signal. A high-resolution Charged Coupled Device (CCD) camera captured a high-resolution image. The cut-off values were previously established using healthy and disease controls (n = 840). For Mi-2β the cut-off was defined as 278 median fluorescence intensity (MFI). To assess clinical specificity for the PMAT assay, a total of 775 disease controls and healthy individuals were tested.

Statistical methods

The data was statistically evaluated using the Analyse-it software (Leeds, UK). For method comparison, data was normalized by dividing patient results by cut-off value. χ², Spearman’s correlation and Cohen’s kappa agreement test were carried out to analyse the agreement between portions and P values < 0.05 were considered significant. Receiver operating characteristics (ROC) analysis was used to analyse the discriminatory ability of different immunoaassays. A Venn diagram was generated using a software library for plotting area-proportional two- and three-way Venn diagrams in Python obtained from the library definition (https://pypi.org/project/matplotlib-venn/).

Results

A total of 29/82 (35.4%) samples were positive for anti-Mi-2β antibodies by LIA and/or PMAT. Of those, 24 (82.8%) were positive by Mi-2β LIA, five (17.2%) by Mi-2α LIA and 23 (79.3%) by Mi-2β PMAT. The comparison shows varying agreement between the different methods as shown by kappa statistics (0.27–0.77). When the results obtained from the LIA were used as the reference for ROC analysis, good discrimination and high area under the curve values were found for both PMAT vs LIA Mi-2α and LIA Mi-2β (Fig. 1). When analysing the results in the context of the myositis phenotype, LIA Mi-2α was positive in 5/57 (8.8%) DM, in 0/16 (0.0%) PM and 9/9 (0.0%) juvenile DM (JDM) patients (Table 1). For LIA Mi-2β, 19/57 (33.3%) DM, 3/16 (18.8%) PM and 2/9 (22.2%) JDM patients were positive. In addition, for PMAT Mi-2α, 21/57 (36.8%) DM, 0/0 (0.0%) PM and 2/9 (22.2%) JDM patients were positive (Table 1).

Of the six LIA Mi-2β positive samples that were negative by the other methods, three were from PM and three from DM patients (Table 2, Fig. 2, Supplementary Fig. S1, available at Rheumatology online). Of the three DM patients, one also had anti-MDA5, anti-PL-12 and anti-Ku antibodies, the remainder were negative for the MSA included in this study. One of the PM patients had anti-Jo-1 antibodies. Of the two remaining patients, one had anti-PM/Scl-75 antibodies and the other patient was negative for other autoantibodies. The two PMAT Mi-2β positive samples that were negative using LIA were from DM patients, one had anti-PM/Scl-75 antibodies, and the other had no detectable additional antibody specificity. All five anti-Mi-2α positive patients had high titres of anti-Mi-2β antibodies using PMAT, but only two were positive by LIA Mi-2β.

When analysing the results of LIA and PMAT in light of IIF ANA results, six samples showed a speckled nuclear pattern that is compatible with the presence of anti-Mi-2 antibodies, one sample had a cytoplasmic pattern and four were negative (Table 2).

Next, we studied the diagnostic utility of the PMAT system using an extended cohort of patients (Fig. 3). The ROC analysis showed an area under the curve of 0.82 (95% confidence interval = 0.77, 0.87). At the previously established cut-off, the sensitivity and specificity were 28.1% and 99.6%, respectively.

Discussion

The frequency of anti-Mi-2 antibodies has been reported to range from 11–59% in adult DM and from 4–10% in paediatric DM [8–11]. Although known and thoroughly studied for more than two decades, anti-Mi-2 antibodies are not included in an IIM diagnostic or classification criteria [2, 3, 5, 16, 20] nor has a anti-Mi-2 immunoassay been approved by the Food and Drug Administration (USA). Thorough evaluation of autoantibody assays for the detection of MSA and MAA is of utmost importance, as some of these antibodies are included or being considered for IIM classification criteria [1, 3–5, 16, 20]. The biomarkers are not only relevant for establishing the diagnosis, but also in stratification of IIM patients into specific disease phenotypes [1, 20]. In addition, most of the clinical associations of MSA and MAA have been established using IP, although multiple-analyte LIA and dot blots (DB) are increasingly being used for the detection of MSA. However, these assays also have limitations including the lack of true quality controls [16], poor sensitivity combined with limited specificity for some analytes and subjectivity in interpretation [21]. To address the subjectivity of interpreting LIA and DB assays, automated digital scanning systems have been developed and introduced for LIA and DB [8, 21]. A semi-quantitative approach using digital scanning systems allows for the analysis of discrepant results considering the antibody levels (titres). Lastly, the results of LIA as have been shown to strongly depend on incubation temperature, especially for negative or low positive samples [9]. Several studies have evaluated LIAs for the detection of MSA [8, 9, 17, 18, 22], but only a few compared the results to IP. The LIA evaluated in the present study includes two isoforms of Mi-2, namely Mi-2α and Mi-2β. Historically, Mi-2β was the only antigen used but eventually, Mi-2α was added to the LIA used in the present study. Consequently, early studies of this assay reported Mi-2 positivity defined on anti-Mi-2β reactivity. More recent studies define Mi-2 positivity either on reactivity to one of the two isoforms, or both reactivities are reported individually.

In the present study, we compared results obtained with a newly developed PMAT to results derived from LIA that was previously evaluated in different studies. In a study by Ghirardello et al. 4% of 208 IIM patients tested positive for Mi-2 antibodies using LIA and 7% using an in-house
laboratory developed test. The specificities were 98% for LIA and 100% for IP [8]. In a more recent study, when three different blot assays for the detection of anti-Mi-2 antibodies were compared, the sensitivity ranged from 4.8 to 6.2% with specificities between 98.7 to 100.0%. Interestingly, the prevalence of anti-Mi-2a antibodies were slightly higher than for anti-Mi-2b antibodies (5.5% vs 4.8%), which is in contrast to our data (6.1% vs 29.3%) [18]. In the study by Cavazzana et al. LIA and IP were compared and 3/57 (5.2%) and 8/57 (14.0%) were found to be positive for Mi-2a/b. The kappa agreement reached 0.5 [17]. A fourth study that evaluated the LIA reported anti-Mi-2 antibodies in different diseases and using different methods.

**TABLE 1** Prevalence of anti-Mi-2 antibodies in different diseases and using different methods

<table>
<thead>
<tr>
<th>Study</th>
<th>Antigen / assay</th>
<th>IIM</th>
<th>DM</th>
<th>PM</th>
<th>JDM</th>
<th>IBM</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mahler et al.</td>
<td>LIA Mi-2a</td>
<td>5/82 (6.1%)</td>
<td>5/57 (8.8%)</td>
<td>0/16 (0.0%)</td>
<td>0/9 (0.0%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>LIA Mi-2b</td>
<td>24/82 (29.3%)</td>
<td>19/57 (33.3%)</td>
<td>3/16 (18.8%)</td>
<td>2/9 (22.2%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>PMAT Mi-2b</td>
<td>23/82 (28.0%)</td>
<td>21/57 (36.8%)</td>
<td>0/16 (0.0%)</td>
<td>2/9 (22.2%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ronnelid et al.</td>
<td>LIA Mi-2</td>
<td>5/153 (3.3%)</td>
<td>4/50 (8.0%)</td>
<td>1/89 (1.1%)</td>
<td>0/4 (0.0%)</td>
<td>0/4 (0.0%)</td>
<td>0/77 (0.0%)</td>
</tr>
<tr>
<td>Cavazzana et al.</td>
<td>LIA Mi-2</td>
<td>3/57 (5.2%)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2016 [17]</td>
<td>IP Mi-2</td>
<td>8/57 (14.0%)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LIA Mi-2a</td>
<td>7/144 (4.8%)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>3/240 (1.3%)</td>
<td></td>
</tr>
<tr>
<td>Vulsteke et al.</td>
<td>LIA Mi-2a</td>
<td>9/144 (6.2%)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1/240 (0.4%)</td>
<td></td>
</tr>
<tr>
<td>2019 [18]</td>
<td>LIA Mi-2(t)</td>
<td>9/144 (6.2%)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1/240 (0.4%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DB Alphadia</td>
<td>8/144 (5.5%)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0/240 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Ghirardello et al.</td>
<td>LIA Mi-2</td>
<td>NP/208 (4%)</td>
<td>NP/65 (12%)</td>
<td>NP/100 (1%)</td>
<td>NA</td>
<td>NP/230 (2%)</td>
<td></td>
</tr>
<tr>
<td>2010 [8]</td>
<td>IP/B Mi-2</td>
<td>NP/208 (7%)</td>
<td>NP/65 (21%)</td>
<td>NP/100 (1%)</td>
<td>NA</td>
<td>0/230 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Zampeli et al.</td>
<td>LIA Mi-2a</td>
<td>6/95 (6.3%)</td>
<td>5/44 (11.4%)</td>
<td>0/29 (0.0%)</td>
<td>0/4 (0.0%)</td>
<td>0/1 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>2019 [19]</td>
<td>LIA Mi-2b</td>
<td>6/95 (6.3%)</td>
<td>6/44 (13.6%)</td>
<td>0/29 (0.0%)</td>
<td>0/4 (0.0%)</td>
<td>0/1 (0.0%)</td>
<td></td>
</tr>
</tbody>
</table>

*Inconsistency between no. and % positive in publication. IIM: idiopathic inflammatory myopathies; JDM: juvenile DM; LIA: line immunoassay; IP: immunoprecipitation; DB: dot blots; PMAT: particle-based multi-analyte technology NA: not analysed; NP: not provided.

In (A-C) using ROC analysis, the discrimination between LIA positive and negative samples is shown for anti-Mi-2b antibodies using the PMAT. Panel (D) shows the levels of antibodies by different assays and panel (E) by myositis diagnosis. LIA: line immunoassay; PMAT: particle-based multi-analyte technology; ROC: receiver operating characteristics.
in 5/153 (3.3%) of IIM patients with a specificity of 100.0% (77 disease controls tested) [9]. In a recent study of a Greek IIM cohort, the prevalence of anti-Mi-2\textsubscript{a} and anti-Mi-2\textsubscript{b} antibodies was 6% for both antibodies. For anti-Mi-2\textsubscript{b} antibodies, all six had a diagnosis of DM and for anti-Mi-2\textsubscript{a}, one patient had amyopathic DM [19]. The significantly higher prevalence of anti-Mi-2 antibodies in our study compared with previous studies can mostly likely be explained by ethnic and geographic differences as it has already been reported that both the prevalence of DM and the of anti-Mi-2 antibodies within DM is significantly higher in Mexican cohorts [14]. This has been attributed to the cellular function of the Mi-2 protein, which can be impacted by ultraviolet light exposure [23]. Interestingly, all of the five anti-Mi-2\textsubscript{a} positive patients as defined by LIA were positive for anti-Mi-2\textsubscript{b} antibodies by PMAT but only two of them were anti-Mi2\textsubscript{b} positive by LIA. Because all of those samples had high levels by PMAT, it is most likely that this difference is based on the different assay characteristics (e.g. immobilization of antigen on a solid phase LIA matrix).

When analysing the discrepant samples in more detail, the analytical (vs ANA IIF) and clinical (form of IIM) are in more agreement with the results obtained using PMAT. In particular, three patients with PM that were only positive using the LIA, tested negative by IIF ANA. The majority of other samples (especially those that were positive by all methods) displayed the expected speckled pattern by IIF [1, 13].

The lack of specific controls for each analyte and proper calibration might represent a technical limitation not only...
for scientific studies performed to date, but also for routine use of the assays. Consequently, studies of run-to-run and also lot-to-lot variability are required to assess the reliability of the assays and to exclude inter-manufacturer variability that may be related to limited precision and reproducibility. Ideally, those studies should contain sufficient samples around the cut-off and follow Clinical and Laboratory Standards Institute guidelines (https://clsi.org/).

Along those lines, a close collaboration between patient groups, research networks [24] and kit manufacturers is mandatory to make serum samples available for calibration and quality control [25, 26]. As it can be challenging to obtain large volume bulk samples, alternative approaches may include pooling of patient samples [27, 28] or the generation of human or humanized monoclonal antibodies.

Conclusions

For the detection of anti-Mi-2 antibodies, overall good agreement was found between LIA and a novel PMAT. Anti-Mi-2β antibodies measured by PMAT tended to be more highly associated with the DM clinical phenotype. Larger multicentre studies are needed to confirm our findings and to compare the results of LIA and PMAT to IP.

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Supplementary data

Supplementary data are available at Rheumatology online.

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