RMD Open
Rheumatic & Musculoskeletal Diseases

ORIGINAL RESEARCH

Predictive value of myositis antibodies: role of semiquantitative classification and positivity for more than one autoantibody

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To cite: Kerola AM, Pietikäinen A, Barantseva J, *et al*. Predictive value of myositis antibodies: role of semiquantitative classification and positivity for more than one autoantibody. *RMD Open* 2025;**11**:e005007. doi:10.1136/rmdopen-2024-005007

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/rmdopen-2024-005007).

Received 16 September 2024
Accepted 11 December 2024

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ABSTRACT

Objectives We assessed the positive predictive value (PPV) of 17 myositis antibodies for having a diagnosis of myositis and other myositis-spectrum conditions (interstitial lung disease (ILD), connective tissue diseases (CTD), malignancy) and evaluated the impact of semiquantitative classification and antibody overlap on the PPVs.

Materials and methods We retrospectively identified 1068 individuals ≥18 years who tested positive for ≥1 antibody in the EUROLINE myositis line blot assay or positive for anti-3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) in an ELISA-based test between 2015 and 2020 in 15 out of the 20 hospital districts in Finland. We extracted clinical diagnoses from the Care Register for Health Care between January 2013 and June 2022.

Results The PPV for a myositis diagnosis (ever during data collection) was highest for anti-HMGCR antibodies (94%), followed by anti-MDA5, anti-Jo-1 and anti-TIF1-γ (49–54%). Regarding other myositis antibodies, 18–42% of cases had myositis. Anti-synthetase antibodies, anti-MDA5, anti-PM-Scl100, anti-SAE1 and anti-Ro52 had a PPV for ILD of 25–47%. A PPV for CTD was highest for anti-Ro52 (57%). The PPV for malignancy was highest for anti-TIF1-γ (38%), followed by anti-PL-7 (32%). Stronger antibody band intensity was associated with higher PPVs for myositis and CTD but not for ILD or malignancies. Simultaneous positivity for ≥2 antibodies compared with single antibody was associated with higher PPVs for myositis, CTD and ILD.

Conclusion The PPV of myositis antibodies for diagnoses of myositis or other myositis spectrum diseases vary considerably between individual autoantibodies. Higher PPVs can be expected with stronger band intensities and with the presence of ≥2 overlapping myositis antibodies.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Myositis antibodies are useful tools for diagnosis of myositis and stratifying the risk of associated conditions such as interstitial lung disease (ILD) and malignancy.

⇒ However, false-positive rates may be high when these tests are used in the context of diagnostic work-up, and more data are needed on the diagnostic accuracy of individual myositis antibodies.

WHAT THIS STUDY ADDS

⇒ A large cohort of patients with a positive myositis antibody result (1068 individuals) were explored for relevant myositis-spectrum diagnoses.

⇒ The PPV for a myositis diagnosis was highest for anti-HMGCR antibodies (94%), followed by anti-MDA5, anti-Jo-1 and anti-TIF1-γ (49–54%), followed by other myositis antibodies (18–42%).

⇒ Anti-synthetase antibodies, anti-MDA5, anti-PM-Scl100, anti-SAE1 and anti-Ro52 had a positive predictive value (PPV) for ILD of 25–47%, and the PPV for malignancy was highest for anti-TIF1-γ (38%), followed by anti-PL-7 (32%).

⇒ Stronger antibody band intensity and simultaneous positivity for ≥2 myositis antibodies compared with single antibody positivity may increase the PPVs of selected myositis antibodies.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ To avoid overdiagnosis of myositis-spectrum disorders, clinicians need to recognise that the PPVs of many myositis antibodies to detect myositis are low, although they are higher when also other myositis-spectrum phenotypes are considered.

⇒ Higher PPVs for myositis can be expected with anti-HMGCR antibodies, stronger antibody band intensities and simultaneous positivity for multiple myositis antibodies

INTRODUCTION

The spectrum of idiopathic inflammatory myopathies (IIMs) comprises multiple clinical subsets including anti-synthetase syndrome, dermatomyositis, immune-mediated necrotising myopathy and sporadic inclusion body myositis.[1] Myositis may also occur as a manifestation of connective tissue diseases (CTDs), such as systemic sclerosis, systemic lupus erythematosus (SLE) or Sjögren's

syndrome, or as a part of an overlap syndrome (a patient meeting classification criteria for at least two CTDs). The key clinical features of myositis-spectrum disorders include skin, muscle, and lung involvement and associated malignancy.[2] Moreover, myositis-spectrum disorders may also manifest without actual myositis: for example, dermatomyositis may be amyopathic, and anti-synthetase syndrome may present with any combinations of myositis, interstitial lung disease (ILD), joint involvement and/or skin involvement.

Myositis antibodies can be used to identify relatively homogeneous subsets of myositis and to predict clinical features, especially the risk of ILD or associated cancer.[2] Autoantibodies associated with myositis-spectrum diseases can be classified as myositis-specific autoantibodies (MSAs) or myositis-associated autoantibodies (MAAs). The former are more specific for IIMs, whereas the latter may be found in other rheumatic conditions as well. The detection of more than one MSA in the same individual is rare, but MAAs, especially anti-Ro52, co-occur with other myositis antibodies more often.[2]

Commercial line blot assays are globally used to detect myositis antibodies to inform clinical decision-making.[3] Thus, knowledge on the reliability of these assays is vital. Recent studies indicate that the diagnostic performance of myositis autoantibody line blot testing may be relatively low with a positive predictive value (PPV) of 63% or lower.[4–6] The PPV seems to be higher for moderate-positive and strong-positive compared with weak positive myositis antibody titres.[6] Prior studies have, however, been fairly small with less than a hundred antibody-positive individuals.[4–6] A larger retrospective study of 242 myositis-antibody positive individuals showed that the PPV across myositis antibodies may vary largely (between 0.0% and 72.7%), but even this study was limited by the low number of individuals positive for some myositis antibodies (n<20 for seven antibodies).[7] Thus, more large-scale studies assessing the diagnostic performance of each myositis antibody individually are warranted.

In this retrospective study, we explored the clinical diagnoses given to adults who tested positive for at least one of 17 myositis antibodies. We assessed the PPVs for diagnoses of myositis, CTDs, ILD and recent cancer. Furthermore, we assessed the impact of semiquantitative classification of myositis antibodies and antibody overlap on the PPVs.

METHODS

Study population

We retrospectively identified all individuals ≥18 years who tested positive for at least one antibody in a myositis line blot immunoassay (LIA; EUROLINE myositis line blot, Autoimmune Inflammatory Myopathies 16 Ag, Euroimmun, Lübeck, Germany) or positive for anti-3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) antibody in a commercial ELISA test (INOVA QUANTA Lite HMGCR ELISA, San Diego, CA, USA) analysed in Turku University Hospital Laboratories (TYKS Laboratories; Turku, Finland) between 1 January 2015 and 31 December 2020. During the study period, this was the only clinical laboratory performing these tests in our country. We included the test results ordered by 15 out of 20 Finnish public healthcare providers, because we did not receive data on clinical diagnoses from the five remaining public healthcare providers (online supplemental table S1).

The indication for antibody testing could not be determined. We included tests done before and after the initiation of immunosuppressive treatment. If a single patient underwent repeat testing, we considered the result positive if any of the tested samples was positive.

The LIA assay included 12 MSAs (anti-Mi-α, anti-Mi-β, anti-melanoma differentiation-associated protein 5 (MDA5), anti-transcription intermediary factor 1 (TIF1)-γ, anti-nuclear matrix protein 2 (NXP2), anti-small ubiquitin-like modifier-1 activating enzyme (SAE1), anti-histidyl transfer ribonucleic acid (tRNA) synthetase (Jo-1), anti-threonyl-tRNA synthetase (PL-7), anti-alanyl-tRNA synthetase (PL-12), anti-glycyl-tRNA synthetase (EJ), anti-isoleucyl-tRNA synthetase (OJ), anti-signal recognition particle (SRP)) and four MAAs (anti-polymyositis (PM)-Scl-100, anti-PM-Scl-75, anti-Ku72/86 and anti-Ro52).

During the entire study period, LIA results were semiquantitatively categorised as weak positive and definite positive, and this information was available for all patients. Weak positive results were included in the total cohort. Anti-HMGCR antibodies were only reported as definite positive. A patient was considered to have overlapping myositis antibodies if they tested positive for ≥2 individual myositis antibodies (either in the same LIA, in repeat LIA testing, or in LIA and anti-HMGCR antibody ELISA test).

Since 2017, band intensity was quantitatively measured for all sera tested with the LIA using automated evaluation of band intensity (EUROLineScan, Euroimmun, Lübeck, Germany). Quantitative results (in densitometry units) were stratified by band intensity as negative (0–10), weak-positive (11–25), moderate-positive (26–50) or strong-positive (>50) according to the manufacturer's guideline. In case of repeat testing, the strongest band intensity was used.

Anti-HMGCR antibodies were analysed with quantitative ELISA but reported only qualitatively according to the threshold value recommended by the manufacturer.

Outcomes

From the Care Register for Health Care (maintained by the Finnish Institute for Health and Welfare), we extracted clinical diagnoses (International Classification of Diseases, 10th revision (ICD-10) codes) of all healthcare contacts in specialised medical healthcare services for the study population between 1 January 2013 and 30 June 2022 from 18 healthcare providers in Finland. These included 15 out of the 20 public healthcare

providers in Finland, and three private healthcare providers (online supplemental table S1). These public healthcare providers covered 227 of the 293 municipalities in Finland in 2022.

Clinical diagnoses were identified if they were registered at any time point during the data collection period (before or after myositis antibody testing). The presence of myositis was identified with the ICD-10 codes G72, G73.7, M33, M36.0, M60.8 and M60.9. The presence of ILD was identified with ICD-10 code J84. Relevant CTDs (SLE, systemic sclerosis, Sjögren's syndrome, MCTD, and other or unspecified CTDs) were identified by ICD-10 codes M32, M34, M35.0, M35.1, M35.8 and M35.9. We also studied a combined endpoint of myositis-spectrum disorders, comprising of any of the following diagnoses: myositis, ILD or CTD. Malignancies were identified only if they were registered within ±3 years from the date of the myositis antibody testing (ICD-10 codes C00–C99).

The use of the following systemic medications were extracted from the Care Register for Health Care and the Register of Primary Health Care Visits: methylprednisolone, prednisolone, cyclophosphamide, immunoglobulins, methotrexate, rituximab, mycophenolate mofetil, tofacitinib, ciclosporin, tacrolimus, azathioprine. We lacked data on hydroxychloroquine use.

Statistical analysis

The PPVs for the diagnoses of interest were calculated as (100 * number of true positives/number of individuals tested positive). In the main analysis, PPVs for myositis, CTD, ILD, a combined event 'myositis, CTD or ILD' and malignancy were calculated for being positive for any myositis antibody. In a sensitivity analysis, we excluded those who were only anti-Ro52 positive. PPVs were also calculated for each myositis antibody separately. PPVs for diagnosing myositis and any of the triad 'myositis, ILD or CTD' were calculated according to the semiquantitative antibody classification (weak positive or definite positive), according to the quantitative antibody results (band intensity weak, intermediate or strong) and according to the presence of overlapping myositis antibodies (single autoantibody positivity or ≥2 overlapping antibodies). The statistical significance of difference between groups was evaluated by Fisher's exact test or Freeman-Halton extension of Fisher's exact test, whichever appropriate. The differences in quantitative antibody band intensities between the clinically true- and false-positive cases were evaluated by unpaired t-tests. All statistical analyses were conducted with R V.4.3.0, R Foundation for Statistical Computing, Vienna, Austria (https://www.R-project.org/).

Ethics statement

The data on the myositis antibodies and the clinical diagnoses and medications were joined and pseudonymised by Finnish Social and Health Data Permit Authority (Findata; approval THL/483/14.02.00/2021). In addition, all the included hospital districts gave consent for the use of their data. Ethical board review and informed consent were waived by law due to the study design. The participants were not contacted. To ensure the anonymity of the results, the minimum number of individuals in reported observations was set to be 3, which is allowed for studies on rare conditions as per Findata's regulations. Observations of 0–2 individuals are hence not reported.

RESULTS

Baseline characteristics

We identified 1406 positive myositis antibody findings among 1068 unique patients (with one or more positive myositis antibodies). Of them, 656 (61.4%) were female, and mean (SD) age was 60.2 (16.2) years. Anti-Ro52, anti-PM-Scl75, anti-SRP, anti-Ku72/86, anti-PL-7 and anti-Jo-1 were the most frequently detected antibodies (table 1).

Antibody overlap

In total, 801 (75.0%) tested positive for a single antibody, 213 (19.9%) tested positive for two antibodies, and 54 (5.1%) tested positive for ≥3 antibodies. A total of 574 (53.7%) individuals tested positive for at least one MSA, 693 (64.9%) for at least one MAA and 199 (18.6%) had both MSAs and MAAs. Antibodies with the highest rates of overlap with other myositis antibodies were anti-Mi-2α, anti-MDA-5, anti-Jo-1 and anti-PL-12, with over 60% overlap with other myositis antibodies (table 1). Anti-Ro52 overlapped most frequently with other antibodies: more than 40% of patients positive for anti-Jo-1, anti-PL-12 and anti-MDA5 were also anti-Ro52 positive (table 1). Of the 574 individuals with at least one MSA, 62 (10.8%) were positive for another MSA. Detailed information on myositis antibody overlap is presented in online supplemental table S2.

Semiquantitative and quantitative antibody band intensities

Of the 1406 positive antibody findings, 995 (70.8%) were definite positive and 411 (29.2%) weak-positive. Of the 1068 patients, 786 (73.6%) had at least one definite positive antibody (with or without other weak positive findings), and the remaining 282 (26.4%) had only weak-positive findings. The percentage of patients defined as weak-positive varied from 8.8% (anti-Jo-1) to 62.5% (anti-Mi-2α) (table 1).

The quantitative analysis of band intensities was carried out for all individuals who tested positive for any of the antibodies in the LIA between 2017 and 2020 (570 unique patients). Of the 814 quantitative results, 282 (34.6%) were strong positive, 158 (19.4%) moderate positive and 374 (45.9%) weak positive. Out of the 570 individuals with quantitative results, 231 (40.5%) had ≥1 strong positive antibody finding, 125 (21.9%) had ≥1 moderate positive antibody finding without strong positive findings, and 214 (37.5%) had only weak positive antibody findings. For most antibodies, weak band intensities were most common, but for some (anti-Jo-1,

Table 1 Characteristics of individuals positive for each myositis-associated or myositis-specific antibody and overlap with other antibodies

	N	Female, n (%)	Age in years, mean (SD)	Weak-positive, n (%)	Overlap with Ro52, n (%)	Overlap with any antibody, n (%)
Myositis-associated antibodies						
Ro52	444	296 (66.7)	59.1 (15.9)	46 (10.4)	NA	173 (39.0)
PM-Scl75	140	92 (65.7)	54.0 (17.3)	51 (36.4)	16 (11.4)	49 (35.0)
Ku72/86	93	55 (59.1)	60.9 (17.4)	35 (37.6)	13 (14.0)	38 (40.9)
PM-Scl100	74	40 (54.1)	60.4 (16.7)	23 (31.1)	10 (13.5)	29 (39.2)
Myositis-specific antibodies						
Anti-synthetase antibodies						
PL-7	93	49 (52.7)	65.1 (13.5)	38 (40.9)	19 (20.4)	34 (36.6)
Jo-1	91	63 (69.2)	55.6 (17.0)	8 (8.8)	49 (53.8)	58 (63.7)
PL-12	36	22 (61.1)	61.9 (15.2)	13 (36.1)	16 (44.4)	22 (61.1)
EJ	16	9 (56.3)	63.0 (13.0)	4 (25.0)	3 (18.8)	7 (43.8)
OJ	12	8 (66.7)	67.6 (9.7)	5 (41.7)	< 3	7 (58.3)
Other						
SRP	121	66 (54.5)	62.1 (15.7)	74 (61.2)	24 (19.8)	55 (45.5)
TIF1-γ	71	46 (64.8)	64.5 (13.7)	21 (29.6)	13 (18.3)	23 (32.4)
Mi-2-β	61	33 (54.1)	61.7 (15.8)	29 (47.5)	5 (8.2)	23 (37.7)
SAE1	41	27 (65.9)	61.5 (15.5)	18 (43.9)	12 (29.3)	23 (56.1)
NXP2	32	20 (62.5)	61.5 (15.0)	13 (40.6)	5 (15.6)	17 (53.1)
Mi-2α	32	23 (71.9)	62.6 (14.7)	20 (62.5)	6 (18.8)	22 (68.8)
MDA5	31	18 (58.1)	56.2 (14.8)	12 (38.7)	13 (41.9)	20 (64.5)
HMGCR	18	9 (50.0)	66.2 (14.7)	NA	< 3	5 (27.8)

Percentages are given as percentages of the total number of patients positive for each antibody.
EJ, glycyl-tRNA synthetase; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; Jo-1, histidyl transfer ribonucleic acid (tRNA) synthetase; MDA5, melanoma differentiation-associated protein 5; NXP2, nuclear matrix protein 2; OJ, isoleucyl-tRNA synthetase; PL-7, threonyl-tRNA synthetase; PL-12, alanyl-tRNA synthetase; PM, polymyositis ; SAE1, small ubiquitin-like modifier-1 activating enzyme; SRP, signal recognition particle; TIF1, transcription intermediary factor 1.

Table 2 Number of individuals with outcomes and PPVs (as percentages) with 95% CIs among those having at least one positive myositis antibody (any myositis antibody) and among those having at least one positive myositis antibody other than anti-Ro52 (sensitivity analysis)

	All (n=1068)		Excluding patients with only anti-Ro52 (n=797)		
	n	PPV (95% CI)	n	PPV (95% CI)	P
Myositis	298	27.9 (25.3, 30.7)	254	31.9 (28.7, 35.2)	0.063
ILD	220	20.6 (18.3, 23.1)	179	22.5 (19.7, 25.5)	0.333
CTD	368	34.5 (31.7, 37.4)	204	25.6 (22.7, 28.7)	< 0.0001
Myositis, ILD or CTD	686	64.2 (61.3, 67.1)	482	60.5 (57.0, 63.8)	0.100
Malignancy	200	18.7 (16.5, 21.2)	153	19.2 (16.6, 22.1)	0.811
Glucocorticoids or other systemic medications	686	64.2 (61.3, 67.1)	498	62.5 (59.1, 65.8)	0.466

CTD, connective tissue disease; ILD, interstitial lung disease; PPV, positive predictive value.

anti-Ro52), strong band intensities were predominant (online supplemental table S3).

Positive predictive value (PPVs) of positive myositis antibodies

The PPV of having any positive myositis antibody was 27.9% for myositis, 20.6% for ILD, 34.5% for CTD and 64.2% for 'myositis, ILD or CTD' (table 2). The PPV for malignancy within +/-3 years of myositis antibody testing was 18.7%. A total of 64.2% of cases had used glucocorticoids or other systemic medications during the follow-up. In 442 (41.4%) cases, the initiation of immunosuppression preceded the antibody testing. In a sensitivity analysis excluding 271 individuals positive only for anti-Ro52 (n=797), the PPVs remained similar except for lower PPV for CTDs (table 2).

The PPV for a diagnosis of myositis was highest for anti-HMGCR antibodies (94%), followed by anti-MDA5, anti-Jo-1 and anti-TIF1-γ (49–54%) (figure 1A). For the other myositis autoantibodies, only 18–42% of patients had a myositis diagnosis (figure 1A). For anti-synthetase antibodies (anti-Jo-1, anti-PL-7, anti-PL-12 and anti-EJ), the PPV for ILD was 25–47%. The PPV for ILD was also notable for anti-MDA5, anti-PM-Scl100, anti-SAE1 and anti-RO52 antibodies (25–29%) (figure 1B). A CTD diagnosis was especially common in individuals with anti-Ro52 antibodies (57%), while other myositis antibodies showed PPVs for CTDs between 16% and 40% (figure 1C). The PPVs for 'myositis, ILD, or CTD' were 100% for anti-HMGCR, 81–82% for anti-Jo-1 and anti-Ro52, and 50–69% for the rest of the antibodies (figure 1D). The majority of patients in all of the antibody-positive subgroups had received glucocorticoids or other systemic medications during the follow-up (53–84%) (figure 1E). Malignancy within +/-3 years of myositis antibody testing was detected most often in anti-TIF-1-γ positive individuals (38%), followed by anti-PL-7 positive individuals (32%) (figure 1F).

Impact of antibody band intensity on positive predictive values (PPVs)

Definite- versus weak-positive results

The PPV of definite positive myositis antibody findings compared with the PPV of weak positive findings was higher for myositis, CTD and the composite endpoint 'myositis, ILD or CTD', but not for ILD alone or malignancy (table 3).

Subgroup analyses comparing PPVs for definite and weak positive results were conducted for most antibodies (anti-EJ, anti-OJ and anti-Jo-1 were not analysed due to low numbers). In most subgroups, numerically higher PPVs for myositis were detected for a definite positive than a weak-positive result, but the differences were statistically significant only in anti-SAE1 and anti-SRP positive subgroups (figure 2A).

The PPVs for 'myositis, ILD or CTD' were statistically significantly higher among those with definite positive antibodies compared with those with weak-positive antibodies in anti-Ro52, anti-MDA5, anti-Ku-72/86, anti-TIF1-γ, anti-SRP and anti-PM-Scl75 positive subgroups (figure 2B).

Strong, moderate- and weak-positive results

Analyses comparing individuals with ≥1 strong positive antibody finding, those with ≥1 moderate positive but no strong positive antibody findings, and those with only weak positive antibodies yielded similar results: band intensity was associated with the PPV for myositis, CTD and 'myositis, ILD or CTD', but not ILD alone nor malignancies (table 3).

We determined the PPVs for the diagnosis of 'myositis' and for the diagnoses 'myositis, ILD or CTD' in the largest myositis autoantibody subgroups by band intensity (figure 3A and B). Band intensity was statistically significantly associated with the presence of myositis in the anti-TIF1-γ and anti-SRP positive subgroups (figure 3A). Band intensity was also associated with the diagnosis of 'myositis, ILD or CTD' in the anti-TIF1-γ anti-SRP, anti-Jo-1 and anti-Ro52 positive subgroups (figure 3B). Distributions of antibody band intensities among cases with myositis compared with those without myositis and cases with and without 'myositis, ILD or CTD' are shown in online supplemental figure S1.

Impact of antibody overlap on positive predictive values (PPVs)

The presence of two or more positive myositis antibodies compared with single antibody positivity was associated with higher PPVs for all studied outcomes except for malignancy (table 3). These findings were even more pronounced for anti-Ro52 overlapping with other myositis antibodies (online supplemental table S4). In subgroups by specific antibodies (anti-EJ and anti-OJ not analysed due to low numbers), patients with ≥2 overlapping myositis autoantibodies had a higher PPV for myositis in the anti-Jo-1, anti-SRP and anti-Ro52 positive groups (figure 4A). The PPV for myositis was also numerically higher for MDA5, SAE1, Pm-Scl100, Pm-Scl75 and Mi-2β together with another antibody compared with single antibody positivity, although these results did not reach statistical significance. The PPV for 'myositis, ILD or CTD' was higher in those with overlapping antibodies in the anti-Jo-1, anti-SRP, anti-Ro52, anti-PM-Scl100, anti-PM-Scl75, anti-PL-7 and anti-Ku-72/86-positive groups (figure 4B).

DISCUSSION

In this observational study of 1068 individuals with positive myositis autoantibodies as detected by a 16-antigen line blot test or a commercial ELISA test for anti-HMGCR antibodies, we explored how many were actually diagnosed with myositis or associated conditions. Our main findings are that large variation exists in the PPVs of individual myositis antibodies and that the PPV for myositis-spectrum conditions improves considerably by semiquantitative classification and simultaneous positivity for more than one myositis antibody. To our knowledge, our study

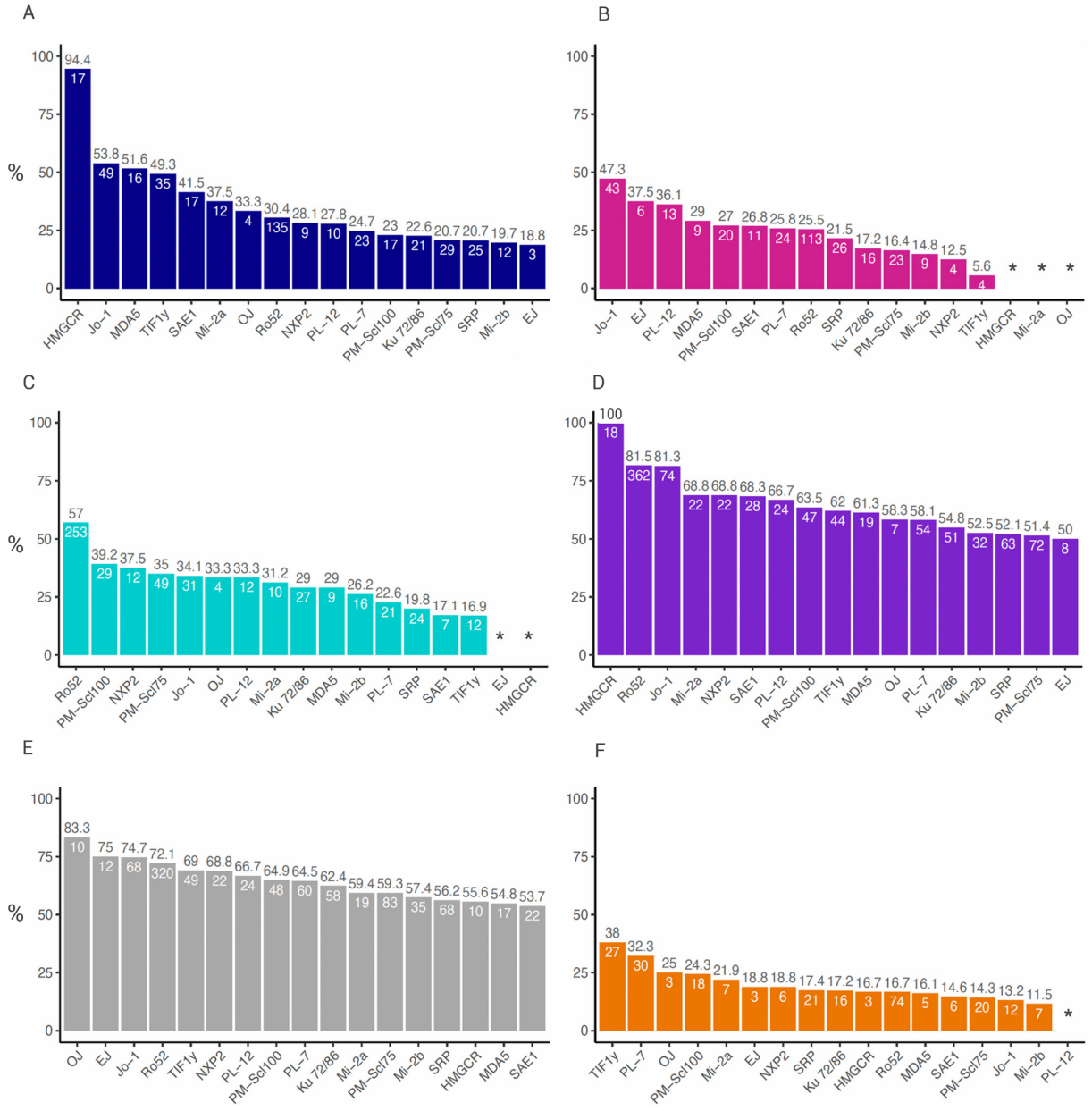

Figure 1 Myositis antibody PPVs for diagnoses of (**A**) myositis; (**B**) ILD; (**C**) CTD; (**D**) myositis, ILD or CTD; (**E**) Use of glucocorticoids (GCs) or other systemic medications; and (**F**) Malignancy +/-3 years from myositis antibody testing. Black numbers show PPVs/percentages, and white numbers show absolute numbers of patients. Asterisks (*) represent 0–2 observations.

CTD, connective tissue disease; EJ, glycyl-tRNA synthetase; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; ILD, interstitial lung disease; Jo-1, histidyl transfer RNA synthetase; MDA5, melanoma differentiation-associated protein 5; NXP2, nuclear matrix protein 2; OJ, isoleucyl-tRNA synthetase; PL-7, threonyl-tRNA synthetase; PL-12, alanyl-tRNA synthetase; PM, polymyositis; PPV, positive predictive value; SAE1, small ubiquitin-like modifier-1 activating enzyme; SRP, signal recognition particle; TIF1γ, transcription intermediary factor 1-γ.

is the largest to date to explore clinical diagnoses among individuals with detectable myositis antibodies, allowing us to examine even the rarer myositis antibodies. In cases where MSA/MAA testing serves the purpose of screening and the clinical pretest probability of a myositis-spectrum disorder is low, our findings may aid clinicians to use caution in the interpretation of positive myositis antibody findings.

Our results are in agreement with earlier studies on the PPV of the EUROLINE myositis antibody LIA, in which the PPVs for IIM diagnoses have ranged between 16% and 63%.[4–9] Myositis antibodies are associated not only with clinical myositis, but also with ILD or skin disease with absent or subclinical myositis.[2] According to a Spanish study among 130 individuals with a positive myositis antibody test in the LIA, the PPV for IIM or

Table 3 Number of individuals with outcomes and PPVs (as percentages) by antibody band intensity (≥1 definite positive antibody vs only weak-positive antibodies and strong vs moderate vs weak-positive antibodies) and by the presence of antibody overlap (≥2 overlapping positive antibodies vs single-positive antibodies)

	n	PPV (95% CI)	n	PPV (95% CI)	n	PPV (95% CI)	P
	≥1 definite positive antibody (n=786)		**Only borderline positive antibodies (n=282)**				
Myositis	256	32.6 (29.4, 35.9)	42	14.9 (11.2, 19.5)			< 0.0001
ILD	171	21.8 (19.0, 24.8)	49	17.4 (13.4, 22.2)			0.123
CTD	337	42.9 (39.5, 46.4)	31	11.0 (7.9, 15.2)			< 0.0001
Myositis, ILD or CTD	572	72.8 (69.6, 75.8)	114	40.4 (34.9, 46.2)			< 0.0001
Malignancy	152	19.3 (16.7, 22.2)	48	17.0 (13.1, 21.8)			0.424
	Strong positive* (n=231)		**Moderate positive† (n=125)**		**Weak positive‡ (n=214)**		
Myositis	78	33.8 (28.0, 40.1)	33	26.4 (19.5, 34.7)	34	15.9 (11.6, 21.4)	< 0.0001
ILD	72	31.2 (25.5, 37.4)	28	22.4 (16.0, 30.5)	48	22.4 (17.4, 28.5)	0.0649
CTD	118	51.1 (44.7, 57.5)	39	31.2 (23.7, 39.8)	26	12.1 (8.4, 17.2)	< 0.0001
Myositis, ILD, or CTD	189	81.8 (76.3, 86.3)	83	66.4 (57.7, 74.1)	97	45.3 (38.8, 52.0)	< 0.0001
Malignancy	42	18.2 (13.7, 23.7)	33	26.4 (19.5, 34.7)	38	17.8 (13.2, 23.4)	0.112
	≥2 overlapping positive antibodies (n=267)		**Single positive antibody (n=801)**				
Myositis	122	45.7 (39.8, 51.7)	176	22.0 (19.2, 25.0)			< 0.0001
ILD	84	31.5 (26.2, 37.3)	136	17.0 (14.5, 19.7)			< 0.0001
CTD	116	43.4 (37.6, 49.4)	252	31.5 (28.3, 34.8)			0.0005
Myositis, ILD, or CTD	213	79.8 (74.6, 84.2)	473	59.1 (55.6, 62.4)			< 0.0001
Malignancy	45	16.9 (12.8, 21.8)	155	19.4 (16.8, 22.2)			0.415

The groups were compared with Fisher's exact test and its Freeman-Halton extension.
*≥1 strong-positive antibody findings.
†≥1 moderate-positive antibody findings without strong-positive antibody findings.
‡Only weak-positive antibody findings without strong or moderate-positive antibody findings.
CTD, connective tissue disease; ILD, interstitial lung disease; PPV, positive predictive value.

other systemic autoimmune diseases compatible with the myositis antibodies was higher (52%) compared with PPV for IIM alone (33%).[4] Our results align with this finding: PPVs for myositis, ILD or CTD were considerably higher compared with PPVs for myositis alone across individual antibodies. Given the heterogeneity of conditions associated with myositis antibodies and the possibility of ILD- or skin-predominant phenotypes, it has been suggested that the term MSA could be replaced with 'myositis-spectrum disease autoantibodies'.[2]

Some prior studies have evaluated PPVs for myositis or related conditions for individual antibodies.[7 9] In a retrospective analysis of 242 patients with ≥1 positive myositis antibody, the PPV for IIM across all antibodies ranged from 0.0% to 72.7%, but the number of patients positive for each individual antibody was rather low (between 1 and 66 cases).[7] The highest PPVs for IIM were found for anti-Mi-2α (8/11 cases, PPV 72.7%), followed by anti-SAE (2/4 cases, PPV 50.0%) and anti-Jo-1 (9/20 cases, PPV 45.0%).[7] For other myositis antibodies, PPVs were below 30%.[7] In a New Zealand–based study on 171 myositis antibody-positive individuals, the PPVs for an expert clinician-confirmed diagnosis of IIM or a phenotype compatible with anti-synthetase syndrome was 0–50% for individual antibodies with a cut-off of 10 densitometry units.[9] Again, the number of patients in the subgroups positive for individual antibodies were small (2–35 patients). In our study, the PPVs for IIM diagnosis varied by antibody and ranged from as high as 94% for anti-HMGCR to 19–54% for antibodies detected in the LIA, when also weak-positive results were considered. When only definite positives in the LIA were considered, the PPVs for IIM diagnosis slightly improved to 22–63%. To avoid overdiagnosis of IIMs, antibody-specific PPVs should be considered when interpreting each positive MSA/MAA result.

Previously, ILD has mostly been linked to anti-synthetase, anti-MDA5, anti-Ro-52 and anti-PM-Scl antibodies.[10 11] Our results showed that in addition to ILD being frequent in cases with any of the abovementioned antibodies (26–47%), more than 20% of patients positive for anti-SRP or anti-SAE were diagnosed with ILD. In the literature, anti-TIF1-γ and anti-NXP2 have been linked to paraneoplastic dermatomyositis.[11 12] In our study, although malignancies were most often detected among cases positive for anti-TIF1-γ (in 38%), they were

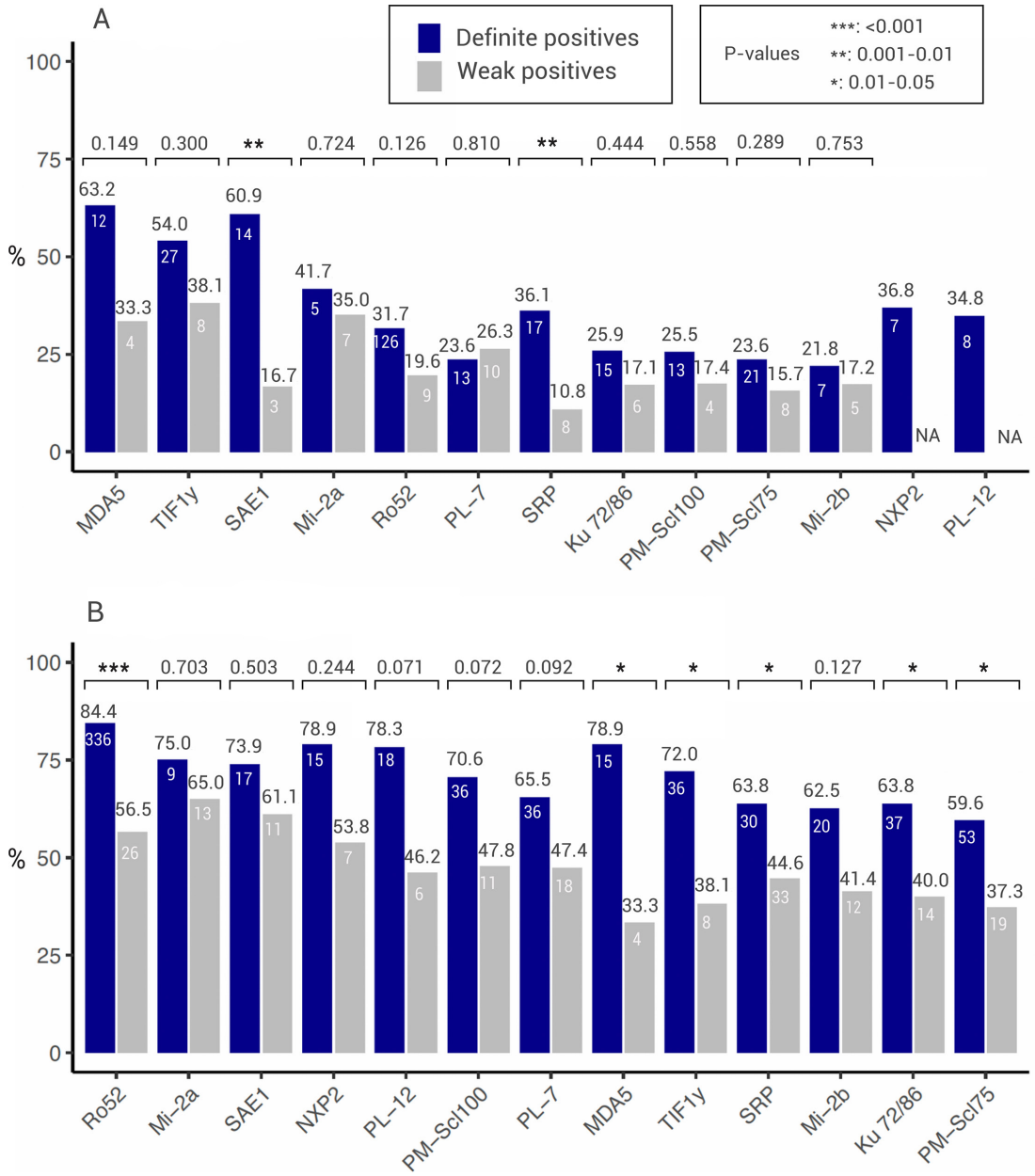

Figure 2 Myositis antibody PPVs for diagnoses of (**A**) myositis and **(B)** myositis, ILD or CTD separately for definite positive and weak-positive antibody testing. Black numbers above the bars show PPVs/percentages, and white numbers on the bars show absolute numbers of patients. 'NA" represents 0–2 observations. The statistical significance of the association between positive versus weak-positive antibody result and the outcome was evaluated by Fisher's exact test.
CTD, connective tissue disease; ILD, interstitial lung disease; MDA5, melanoma differentiation-associated protein 5; NXP2, nuclear matrix protein 2; PL-7, threonyl-tRNA synthetase; PL-12, alanyl-tRNA synthetase; PM, polymyositis; PPV, positive predictive value; SAE1, small ubiquitin-like modifier-1 activating enzyme; SRP, signal recognition particle; TIF1y, transcription intermediary factor 1-γ.

quite common in cases positive for antibodies against PL-7, OJ, PM-Scl100, Mi-2a, EJ and NXP2 (18–32%). This is a noteworthy result, because anti-synthetase syndrome and overlap IIM-CTD-associated myositis are currently considered low-risk features in cancer risk stratification.[13] Our findings call for vigilance for symptoms and signs of ILD and cancer in individuals positive for myositis antibodies beyond the well-recognised associations.

With the exception of anti-HMGCR positive individuals of whom 94% had myositis, a considerable proportion of cases with myositis antibodies (especially weak band intensities) were not diagnosed with myositis or even any of the selected myositis-spectrum conditions (myositis, ILD or CTD), indicating a high false–positive rate. Our results align with those of previous studies suggesting that myositis LIAs are widely used in clinical practice for early diagnostic work-up of suspected IIM or differential diagnosis of autoimmune rheumatic diseases and/or ILD rather than for stratification of established IIM.[7] False-positive myositis antibody findings may be common

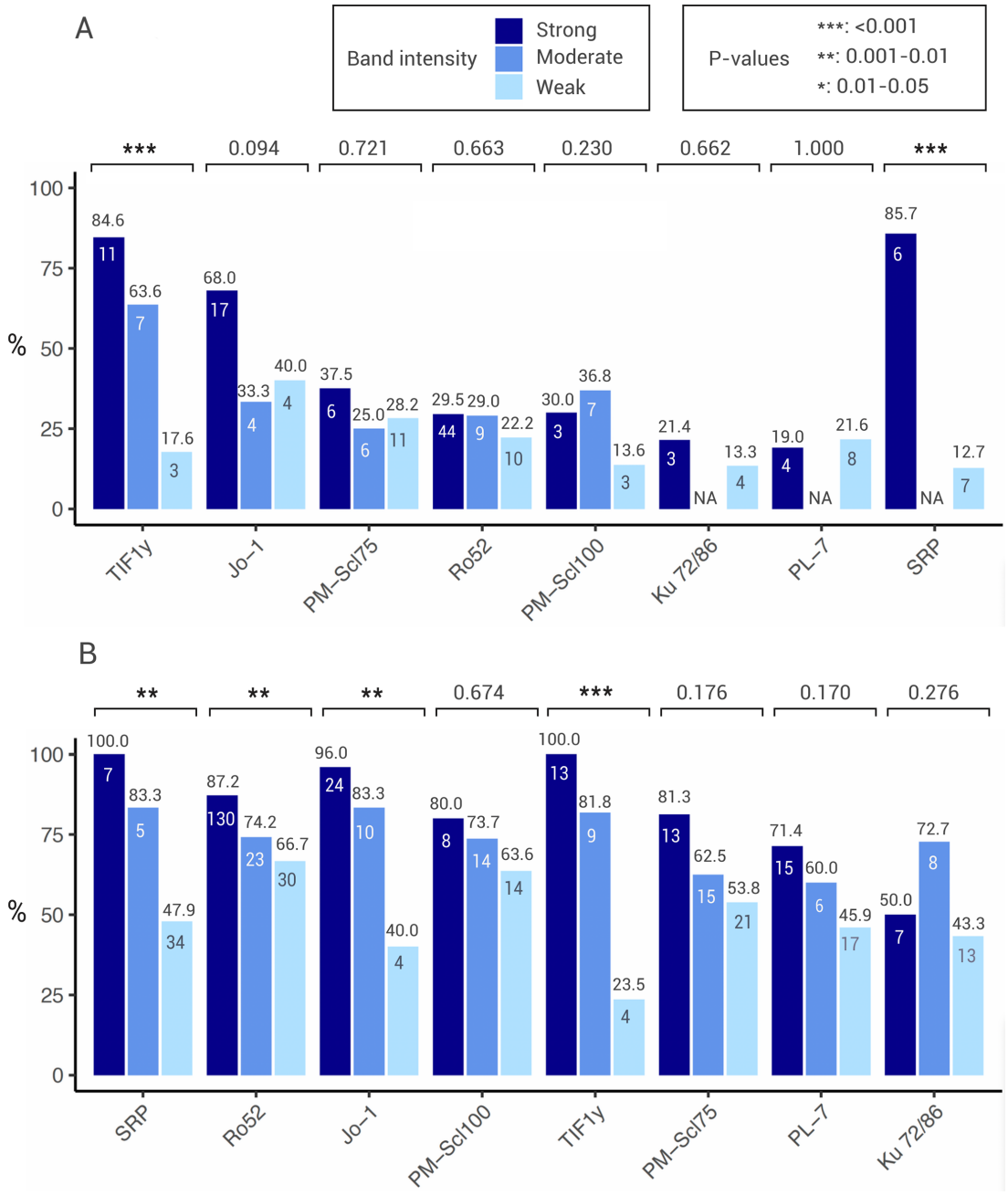

Figure 3 Myositis antibody PPVs by band intensity (strong, intermediate and weak) for diagnoses of (**A**) myositis and (**B**) myositis, ILD or CTD. Black numbers above the bars show PPVs/percentages, and white numbers on the bars show absolute numbers of patients. 'NA' represents 0–2 observations. The statistical significance of the association between band intensity groups and the outcome was evaluated by Fisher's exact test (Freeman-Halton extension).
CTD, connective tissue disease; ILD, interstitial lung disease; Jo-1, histidyl transfer RNA synthetase; PL-7, threonyl-tRNA synthetase; PM, polymyositis; PPV, positive predictive value; SRP, signal recognition particle; TIF1y, transcription intermediary factor 1-γ.

in infectious and post-infectious states,[14 15] but rare in diseases such as in lung and breast cancer.[16 17]

Previous studies have already implied that the PPVs of myositis antibodies may increase with stronger signal intensity, but these studies have been limited by relatively low sample sizes and lack of assessing the impact of signal intensity on the PPVs of individual myositis antibodies.[4–6 8] In our study, PPVs higher than 80% for myositis were detected for strong band intensities of anti-TIF1-γ and anti-SRP. For detecting myositis, ILD or CTD, PPVs were over 80% for strong band intensities of anti-SRP, anti-Jo-1, anti-TIF1-γ, anti-PM-Scl and anti-Ro52. To increase the specificity and PPVs, higher band-intensity cutoffs could be considered for some of these antigens. However, increasing the cut-off level may increase the risk of missing clinically significant cases, making the equilibrium between sensitivity and specificity fragile.[18]

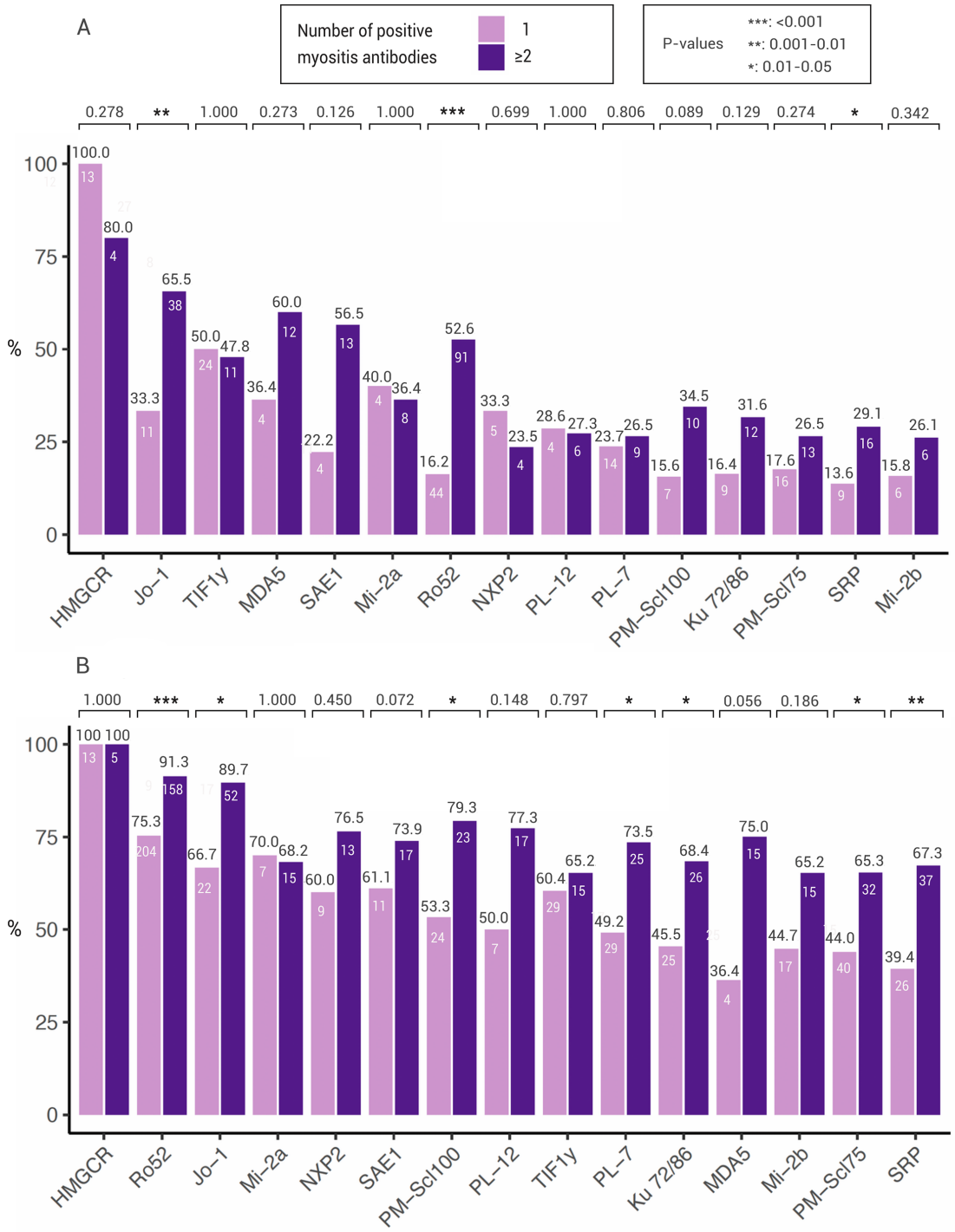

Figure 4 Myositis antibody PPVs by the presence of overlapping myositis antibodies for diagnoses of (**A**) myositis and (**B**) myositis, ILD or CTD. Black numbers above the bars show PPVs/percentages, and white numbers on the bars show absolute numbers of patients. The statistical significance of the association was evaluated by Fisher's exact test. CTD, connective tissue disease; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; ILD, interstitial lung disease; Jo-1, histidyl transfer RNA synthetase; MDA5, melanoma differentiation-associated protein 5; NXP2, nuclear matrix protein 2; PL-7, threonyl-tRNA synthetase; PL-12, alanyl-tRNA synthetase; PM, polymyositis; PPV, positive predictive value; SAE1, small ubiquitin-like modifier-1 activating enzyme; SRP, signal recognition particle; TIF1γ, transcription intermediary factor 1-γ.

This was also corroborated in a study among 171 patients with a positive myositis antibody on Euroline LIA, in which autoantibody-specific cut-offs were created and resulted not only in improved PPVs but also variable increases in false negatives.[9]

Myositis antibodies, especially anti-Ro52, may co-occur in patients with a myositis-spectrum disorder.[2] In a EuroMyositis registry study comprising 1637 adults with confirmed IIM, 84.7% of the autoantibody positive patients had a single autoantibody positivity, and

15.3% had antibodies targeting multiple autoantigens.[19] The occurrence of an MSA or MAA with one or more MAAs was frequent, but the occurrence of more than one MSAs was present in only 0.3% of the autoantibody positive IIM cases.[19] These results are in contrast to our findings showing that over 25% of myositis-antibody–positive individuals had another overlapping myositis antibody. Furthermore, although overlap with Ro52 was most frequent, multiple MSAs also coexisted in 11% of MSA-positive individuals. The EuroMyositis registry study identified autoantibodies by immunoprecipitation, and they studied established IIM patients whereas we studied myositis antibody-positive cases, which may contribute to these contrasting findings.

In anti-synthetase syndrome as well as other rheumatic diseases, the (co-)occurrence of anti-Ro52 predisposes to the development ILD and more severe lung involvement.[20 21] To our knowledge, our study is the first to show that having myositis antibodies against multiple antigens is associated with higher PPVs for myositis, ILD and CTD compared with single antibody specificity. Our findings are in contrast to those of a Spanish study among 130 individuals, in which the probability of clinically false positives (cases without IIM or another phenotype consistent with the myositis antibody) was associated with multiple positive myositis antibodies within one sample, also tested with a LIA.[4]

The main strength of our study is that the study material comprises the majority of myositis antibody tests analysed in Finland during a 6-year period, and the large sample size allowed us to evaluate also rare myositis antibodies and multiple phenotypes associated with myositis antibodies. As a limitation, the indication for myositis antibody testing was not available, and the clinicians' threshold to order these tests may considerably affect the PPVs. Another limitation is that we extracted clinical diagnoses from healthcare registries and did not validate the diagnoses against medical records. Furthermore, for many of the rare disease phenotypes such as anti-synthetase syndrome or immune-mediated necrotising myopathy, no specific ICD-10 code exists. Similarly, we were not able to determine which patients with a CTD diagnosis had a clinical phenotype compatible with the myositis antibody findings (such as anti-synthetase syndrome, amyopathic dermatomyositis or forme fruste presentations of IIM), and which had a clearly defined alternative diagnosis. We lacked data on clinical variables such as muscle enzyme levels and muscle biopsy findings. One more limitation is the lack of validation of the results of the myositis LIA by immunoprecipitation (which is often considered as the gold standard), although the LIA assay itself is validated against immunoprecipitation by the manufacturer. The concordance rate between LIAs and immunoprecipitation has been shown to be moderate or high for the most prevalent MSAs, although line blots may not reliably detect anti-TIF1-γ and rarer anti-synthetase antibodies.[22 23] Despite this, we believe that our results accurately reflect clinical practice, since it is not common to validate the LIA results by immunoprecipitation in routine clinical work. Finally, as in all similar studies, confirmation bias may affect the obtained results: clinicians may be more prone to establish a diagnosis of myositis or CTD in the presence of myositis antibodies, especially high titres.

In conclusion, with the exception of anti-HMGCR positive cases of whom 94% had myositis, a considerable proportion of cases with detectable myositis antibodies are not diagnosed with myositis. Most patients, however, have either myositis, ILD or CTD, emphasising the heterogeneity of conditions associated with myositis antibodies. Malignancies and ILD were detected relatively often in individuals positive for myositis antibodies beyond the well-recognised associations. Stronger antibody band intensity and the presence of myositis antibodies against multiple autoantigens may improve the PPVs, at least for some myositis antibodies.

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Acknowledgements We thank the participating regional registries for retrieving diagnoses for their patients. We thank Findata, Finnish Institute for Health and Welfare, and Auria Biobank for providing platforms to analyse results in data protection-safe environments.

Contributors AMK contributed to the design of the study, made most data analyses, drafted the first version of the manuscript, was responsible for the revisions to the manuscript and is the guarantor of this work. APi and APa contributed to data acquisition and interpretation of the study and critically revised the manuscript. JB contributed to the interpretation of the study and critically revised the manuscript to add intellectual content. AH acquired permissions to conduct the study; contributed to the design, data acquisition, analysis and interpretation; and critically revised the manuscript. All authors approved the final version of the manuscript.

Funding The costs of the study were covered by the Diagnostic Laboratory Division of the South-West Finland's Well-Being District.

Competing interests AMK has received speaker fees from Boehringer-Ingelheim, AbbVie and Sanofi; has participated in the advisory boards of Pfizer and Boehringer-Ingelheim; and has received congress sponsorship from AbbVie and Johnson & Johnson, which are all unrelated to this work. AH has received a speaker fee from UCB Pharma and consulting fees from Labquality Oy, unrelated to this work. APi, JB, APa: none declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants, but the data on the myositis antibodies and the clinical diagnoses and medications were joined and pseudonymised by Finnish Social and Health Data Permit Authority (Findata; approval THL/483/14.02.00/2021). In addition, all the included hospital districts gave consent for the use of their data. Ethical board review and informed consent were waived by law due to the study design. The participants were not contacted. To ensure the anonymity of the results, the minimum number of individuals in reported observations was set to be 3, which is allowed for studies on rare conditions as per Findata's regulations. Observations of 0–2 individuals are hence not reported. Informed consent was waived by law due to the study design. The participants were not contacted.

Provenance and peer review Not commissioned; externally peer-reviewed.

Data availability statement No data are available.

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