HHS Public Access

Author manuscript

Clin Exp Rheumatol. Author manuscript; available in PMC 2024 December 25.

Published in final edited form as:

Clin Exp Rheumatol. 2021 ; 39(3): 519–524. doi:10.55563/clinexprheumatol/7kyr5e.

The indirect immunofluorescence assay autoantibody profiles of myositis patients without known myositis-specific autoantibodies

M. Casal-Dominguez[1,2], **I. Pinal-Fernandez**[1,2,3], **K. Pak**[1], **A. Marin-Sanchez**[4], **M.T. Sanz-Martinez**[4], **A. Baucells**[5], **Y. Hosono**[1], **L. Christopher-Stine**[2], **A.L. Mammen**[1,2], **Johns Hopkins Myositis Center Group**

[1]National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD;

[2]Johns Hopkins University School of Medicine, Baltimore, MD, USA;

[3]Faculty of Health Sciences and Faculty of Computer Science, Multimedia and Telecommunications, Universitat Oberta de Catalunya, Barcelona;

[4]Vall d'Hebron Hospital, Barcelona;

[5]Sant Pau Hospital, Barcelona, Spain.

Abstract

Objective—The indirect immunofluorescence assay (IIFA) is used to screen for the presence of autoantibodies. Our objective was to determine the prevalence and clinical features of IIFA positive myositis patients without known myositis-specific autoantibodies (MSA).

Methods—Sera from healthy comparators (HC) and patients with dermatomyositis (DM), inclusion body myositis (IBM), and polymyositis (PM) with no detectable MSA were tested by IIFA on HEp-2 cells. The pattern of positivity was classified according to the International Consensus on Antinuclear Antibody Patterns. The prevalence and frequency of each IIFA pattern were compared between the different groups.

Please address correspondence to: Andrew L. Mammen or Maria Casal-Dominguez, Muscle Disease Unit, Laboratory of Muscle Stem Cells and Gene Regulation, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, 50 South Drive, Room 1141, Building 50, MSC 8024, Bethesda, MD 20892, USA. andrew.mammen@nih.gov, maria.casal-dominguez@nih.gov.

Maria Casal-Dominguez, MD, PhD*
Iago Pinal-Fernandez, MD, PhD*
Katherine Pak, MD
Ana Marin-Sanchez, BS
Maria T. Sanz-Martinez, BS
Andres Baucells, MD
Yuji Hosono, MD, PhD
Lisa Christopher-Stine, MD, MPH
Andrew L. Mammen, MD, PhD

* These authors contributed equally to this project.

Members of the Johns Hopkins Myositis Center group: Harlan Michelle, Jemima Albayda, Eleni Tiniakou, Julie Paik, Christopher Mecoli, Sonye K. Danoff and Tom Lloyd.

Competing interests: L. Christopher-Stine receives royalties from Inova Diagnostics/RDL laboratories for anti-HMGCR autoantibody testing; the other co-authors have declared no competing interests.

Results—Sera from 100 HC, 71 DM, 53 IBM, and 69 PM subjects were included in the study. The IIFA was positive in 35% HC compared to 66% DM ($p<0.001$), 49% IBM, and 64% ($p<0.001$) PM sera. Among IIFA positive sera, the staining was moderate or intense in 43% HC compared to 79% DM ($p<0.001$) but just 54% IBM, and 52% PM sera. IIFA positivity was predominantly nuclear in all groups (all >69%). The most common pattern in myositis patients was fine speckled with no differences between groups. In general, IIFA positive and negative DM patients showed similar clinical features and disease activity.

Conclusion—Half of MSA-negative DM patients have moderate/strong IIFA positivity, predominantly with a fine speckled pattern. In contrast, MSA-negative PM, IBM, and healthy comparators are more often weakly positive for IIFA. These findings suggest that unidentified autoantibodies are more likely to exist in DM patients than in the other myositis groups.

Keywords

indirect immunofluorescence assay; autoimmune myopathies; myositis-specific autoantibodies; immunofluorescence pattern

Introduction

Antinuclear antibodies were first described as a heterogeneous group of antibodies targeting components of the cell nuclei (1) using frozen sections of animal organs as a substrate (2). Currently, the gold standard technique for antinuclear antibody detection is indirect immunofluorescence (IIFA) on Hep-2 cells (3, 4), an epithelial cell line derived from a human laryngeal carcinoma (5). Importantly, IIFA also detects antibodies targeting cytoplasm components and the spindle apparatus (6). Thus, IIFA can be used to detect autoantibodies against all cellular components expressed in Hep-2 cells (7–9). To harmonise IIFA testing and interpretation, a group of experts from different fields and countries created the International Consensus on ANA Patterns (ICAP), designating and describing three major groups of staining categories: nuclear, cytoplasmic, and mitotic (10). Subsequent meetings of the group established a total of 29 distinct IIFA patterns (7). The detection of antibodies by IIFA has diagnostic utility for several autoimmune diseases (11–13).

The idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of diseases characterised by muscle weakness and inflammatory infiltrates on the muscle biopsy. In addition to muscle involvement, the lung, skin, and joints may also be affected (14). The most common types of IIM are dermatomyositis (DM), immune-mediated necrotising myopathy (IMNM), sporadic inclusion-body myositis (IBM), overlap myositis (including antisynthetase syndrome), and polymyositis (PM)(14–16). Importantly, ~ 70% of IIM patients have myositis-specific autoantibodies (MSAs) targeting nuclear and cytoplasmic proteins (17, 18) (19). However, it is unknown whether the remaining 30% of patients have an as of yet unknown MSA.

When tested by IIFA, between 50 to 80% of patients with myositis are positive (20). However, the prevalence of such positivity has not been systematically studied in myositis patients who are negative for known MSAs. Thus, the objective of the present study is

to determine the prevalence and pattern of IIFA positivity in healthy comparators and MSA-negative DM, IBM, and PM patients.

Material and methods

Patients and healthy comparators

MSA negative patients enrolled in the Johns Hopkins Myositis Center Longitudinal Cohort study between 2006 and 2015 were enrolled in the study. Patient serum samples were screened for anti-HMGCR autoantibodies by enzymelinked immunosorbent assay. Line blotting (EUROLINE myositis profile) and immunoprecipitation from S^{35}-labelled HeLa cell lysates were used to screen for anti-SRP, -Mi2, -NXP2, -TIF1g, -MDA5, -Jo1, -PL7, -PL12, -EJ, -OJ, -SAE, -Ku and -Pm/Scl autoantibodies (21, 22). All the IBM patients were tested for anti-NT5c1A antibodies by ELISA (cN-1A [Mup44, NT5C1A], IgG semiquantitative, EA 1675–4801 G. EUROIMMUN). Each patient was classified as DM, PM, or IBM based on Bohan and Peter (23) and Griggs (24) criteria, respectively. In addition, 100 sera from healthy individuals were used as a comparator group.

Strength was evaluated by the examining physician using the Medical Research Council scale. This scale was transformed to Kendall's 0–10 scale for analysis as previously described (25). Serial strength measurements for each patient were made by the same physician. For analyses, right- and left-side measurements for arm abduction and hip flexion strength were combined and the average was used for calculations (possible range 0–10). Serum creatine kinase (CK) levels were included for the longitudinal analysis if obtained within 6 weeks before or after strength testing. Skin manifestations specific for DM (*i.e.* heliotrope rash or Gottron's sign), symptoms of esophageal involvement, and antisynthetase syndrome-associated clinical features (*e.g.* mechanics hands, Raynaud's phenomenon, arthritis, fever) were documented both retrospectively at the onset of the disease and prospectively at each visit. Interstitial lung disease was defined through a multidisciplinary approach as recommended by the American Thoracic Society (26).

HEp2 indirect immunofluorescence testing

Both myositis patient and comparator sera were tested by HEp2 cell IIFA. Serum diluted 1:80 in phosphate-buffered saline (PBS) was overlaid onto fixed Hep-2 cells (Kallestead Hep-2 Cell Line Substrate, Bio Rad, Inc, Redmond, WA) for 30 minutes at room temperature. Each slide was rinsed once with a stream of PBS and then washed with PBS for 10 minutes. After that, the slides were overlaid with 25 microliters of Fluorescein isothiocyanate (FITC) conjugated antiserum to human immunoglobulins (IgG, IgA, IgM, Kallestad FITC conjugate, ref #30446) and incubated for 20 minutes at room temperature. The slides were rinsed briefly with PBS and incubated with 3 drops of Evan's blue counterstaining diluted in 150 ml of PBS for 10 minutes. After that, slides were drained briefly, a coverslip was placed over each slide. The serum dilution 1:80 was chosen as a compromise dilution that will allow us to detect the Hep-2 cells IIFA patterns clearly and has shown a relatively low rate of positivity in healthy comparators (13.4%) (27–30).

Imaging acquisition and reading

The slides were read using a fluorescence microscope (Leica 6000) at X20, X40, and X100 power. The images were randomised and a database was created to perform a blind reading of the IIFA patterns (10). Three different Immunologists (AM, MTS, and AB) read the slides and classified them into negative or positive. If positive, they indicated the Hep-2 cells IIFA pattern according to the ICAP consensus (7). For the analysis of the different patterns, we considered only those patients who had IIFA patterns agreed upon by at least two readers.

Standard protocol approvals

The study received the approval of the Johns Hopkins and National Institutes of Health Institutional Review Boards.

Statistical analysis

Dichotomous variables were expressed as percentages and absolute frequencies, and continuous features were reported as means and standard deviations (SD). Pairwise comparisons for categorical variables between groups were made using the chi-square test or Fisher's exact test, as appropriate. Student's t-test was used to compare continuous variables among groups. CK, a highly positively skewed variable, was expressed as median, first, and third quartile for descriptive purposes. All statistical analyses were performed using Stata/MP 14.1. A 2-sided p-value of 0.05 or less was considered significant with no correction for multiple comparisons.

Results

Sera from 193 MSA-negative myositis patients (71 DM, 53 IBM, and 69 PM) (mean age 51.3 years [SD 16.6 years], 60% female, 74% white, 12% black, and 13% other races) and 100 healthy comparators (mean age 52.4 years [SD 14 years], 41% female, 61% white, 24% black, and 14% other races) were included in the study. Using a fixed dilution of 1:80, thirty-five percent of the healthy comparators were IIFA positive compared to 66% of DM (p<0.001) 64% of PM (p<0.001), and 49% of IBM patients (Table I). Fortyfive percent of the IBM patients were anti-NT5c1A positive and there was no association between this autoantibody and the IIFA positivity (54% of positive and 37% of negative IIFA IBM patients had anti-NT5c1A autoantibodies [p=0.2]).

Most IIFA-positive DM patients had moderate or intense IIFA positivity (79%, p<0.001) compared to 52% with PM, 54% with IBM and 43% of healthy comparators. In contrast, very few DM patients showed weak IIFA-positivity (21%, p<0.001) compared to 48% of PM, 46% of IBM, and 57% of healthy comparators (Table I).

In most IIFA-positive samples, the pattern was nuclear both for myositis patients and healthy comparators (all >69%). In contrast, 40% of IIFA-positive IBM and 33% of IIFA-positive PM patients had a cytoplasmic pattern but most of them had weak cytoplasmic staining; however, this was not significantly different compared to the rest of the subjects included in the study. Finally, mitotic patterns were rarely noted in any of the groups (Table II). The most common IIFA pattern in myositis patients was the nuclear fine speckled, which was

significantly more prevalent in DM than in the comparators (64% *vs.* 39%, p=0.03) (Table III).

The clinical features, the severity of the muscle weakness and muscle enzyme levels were not significantly different between IIFA-positive and IIFA-negative DM patients (Tables IV and V). Less than one-third of the 14 PM patients with a cytoplasmic IIFA pattern showed the characteristic clinical features of the antisynthetase syndrome (29% had ILD, 29% Raynaud's phenomenon, 21% arthritis, 7.1% fever, and 0% mechanic's hands).

Discussion

In this cross-sectional study of a large cohort of MSA-negative myositis patients, we found that those with DM had a higher prevalence and higher intensity of IIFA-positivity than patients with PM or IBM. We also demonstrated that the nuclear fine speckled pattern was the most common IIFA pattern in MSA-negative myositis patients. Taken together, our results suggest that previously unidentified autoantibodies may be present in MSA-negative myositis patients and that these are likely to target nuclear proteins.

Surprisingly, IBM patients had a relatively high prevalence of IIFA positivity (49%). However, most of these had a weak intensity, suggesting that these autoantibodies may be less relevant than those found in other types of myositis. Also, half of the IBM patients were positive for anti-NT5c1A autoantibodies but there was no association between this autoantibody and the IIFA positivity.

Finally, in PM patients, the prevalence of IIFA-positivity was high (64%), but the staining intensity was often weak. Previous studies have reported that 50 to 80% of IIM patients, including those with MSAs, are IIFA-positive and the most common pattern is the nuclear speckled (20). In our study, we found a similar proportion of IIFA-positive patients among our cohort of MSA-negative myositis patients (61%). Also, we confirmed that the nuclear fine speckled was the most common pattern among autoantibody-negative myositis patients.

The strengths of our study include (a) the large sample of MSA-negative myositis patients verified by multiple testing platforms and healthy comparator groups, (b) the uniform testing methodology, (c) the blinded testing, and (d) the availability of clinical information from patients enrolled in the same single-center cohort. However, our study has several limitations. First, it is possible that our MSA screening methods (*e.g.* the line blot test) were not sufficiently sensitive and that some MSA-positive patients could have been included in the study. Additionally, the screening did not include testing for rare antisynthetase autoantibodies (*e.g.* like anti-KS, -Zo and -Ha) or myositis-associated autoantibodies such as those recognising mitochondrial proteins. Also, as we used a semiquantitative evaluation of the IIFA intensity at a fixed dilution of 1:80 and not a quantitative evaluation of the fluorescence, this may have decreased our ability to find significant differences in some of the less prevalent IIFA patterns. Finally, the positivity rate in healthy comparators was 35%, which is higher than previously reported for healthy comparators at a 1:80 dilution (31–33). In this study, we optimised the technique to obtain high-resolution images to improve the evaluation of the IIFA patterns; this may, in part, explain the unexpectedly high rate of

positivity in healthy controls. Moreover, it is widely recognised that the positivity rate is highly variable depending on other factors, including the cell culture conditions or the slide preparation (34). In this regard, the secondary antibody used in the Kallestad HEp-2 IIFA assay recognised not only IgG but also IgA, and IgM; this could help to explain the high positivity rate we observed relative to some other studies in which only IgG IIFA was detected.

These limitations notwithstanding, our study shows that most MSA-negative DM patients have prevalent and intense IIFA positivity and that additional nuclear autoantigens likely remain to be discovered.

Funding:

this research was supported by the Intramural Research Program of the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health. We are also grateful to Dr Peter Buck, whose generous support made this work possible.

References

1. COONS AH, KAPLAN MH: Localization of antigen in tissue cells; improvements in a method for the detection of antigen by means of fluorescent antibody. J Exp Med 1950; 91: 1–13. [PubMed: 15395569]
2. KUMAR Y, BHATIA A, MINZ RW: Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. Diagn Pathol 2009; 4: 1. [PubMed: 19121207]
3. AMERICAN COLLEGE OF RHEUMATOLOGY. CURRENT PRACTICE ISSUES: ACR TRACKING CONCERNS ABOUT ANA TESTING RESULTS ATLANTA GACOR.
4. MERONI PL, SCHUR PH: ANA screening: an old test with new recommendations. Ann Rheum Dis 2010; 69: 1420–2. [PubMed: 20511607]
5. HAHON N, ECKERT HL, STEWART J: Evaluation of cellular substrates for antinuclear antibody determinations. J Clin Microbiol 1975; 2: 42–5. [PubMed: 818105]
6. AGMON-LEVIN N, DAMOISEAUX J, KALLENBERG C et al. : International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. Ann Rheum Dis 2014; 73: 17–23. [PubMed: 24126457]
7. DAMOISEAUX J, ANDRADE LEC, CARBALLO OG et al. : Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International Consensus on ANA patterns (ICAP) perspective. Ann Rheum Dis 2019; 78: 879–89. [PubMed: 30862649]
8. HEROLD M, KLOTZ W, ANDRADE LEC et al. :International Consensus on Antinuclear Antibody Patterns: defining negative results and reporting unidentified patterns. Clin Chem Lab Med 2018; 56: 1799–802. [PubMed: 29634478]
9. ANDRADE LEC, KLOTZ W, HEROLD M et al. : International consensus on antinuclear antibody patterns: definition of the AC-29 pattern associated with antibodies to DNA topoisomerase I. Clin Chem Lab Med 2018; 56: 1783–8. [PubMed: 29813025]
10. CHAN EK, DAMOISEAUX J, CARBALLO OG et al. : Report of the First International Consensus on Standardized Nomenclature of Antinuclear Antibody HEp-2 Cell Patterns 2014–2015. Front Immunol 2015; 6: 412. [PubMed: 26347739]
11. SOLOMON DH, KAVANAUGH AJ, SCHUR PH; AMERICAN COLLEGE OF RHEUMATOLOGY AD HOC COMMITTEE ON IMMUNOLOGIC TESTING G: Evidence-based guidelines for the use of immunologic tests: antinuclear antibody testing. Arthritis Rheum 2002; 47: 434–44. [PubMed: 12209492]
12. MAHLER M, FRITZLER MJ: The clinical significance of the dense fine speckled immunofluorescence pattern on HEp-2 cells for the diagnosis of systemic autoimmune diseases. Clin Dev Immunol 2012; 2012: 494356. [PubMed: 23304189]

13. SATOH M, TANAKA S, CERIBELLI A, CALISE SJ, CHAN EK: A comprehensive overview on myositis-specific antibodies: new and old biomarkers in idiopathic inflammatory myopathy. Clin Rev Allergy Immunol 2017; 52: 1–19. [PubMed: 26424665]
14. DALAKAS MC: Inflammatory muscle diseases. N Engl J Med 2015; 373: 393–4.
15. LUNDBERG IE, TJARNLUND A, BOTTAI M et al. : 2017 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Adult and Juvenile Idiopathic Inflammatory Myopathies and Their Major Subgroups. Arthritis Rheumatol 2017; 69: 2271–82. [PubMed: 29106061]
16. SENECAL JL, RAYNAULD JP, TROYANOV Y: Editorial: A new classification of adult autoimmune myositis. Arthritis Rheumatol 2017; 69: 878–84. [PubMed: 28382651]
17. NISHIKAI M, REICHLIN M: Heterogeneity of precipitating antibodies in polymyositis and dermatomyositis. Characterization of the Jo-1 antibody system. Arthritis Rheum 1980; 23: 881–8. [PubMed: 7406938]
18. REICHLIN M, MATTIOLI M: Description of a serological reaction characteristic of polymyositis. Clin Immunol Immunopathol 1976; 5: 12–20. [PubMed: 1261095]
19. MCHUGH NJ, TANSLEY SL: Autoantibodies in myositis. Nat Rev Rheumatol 2018; 14: 290–302. [PubMed: 29674612]
20. TARGOFF IN: Laboratory testing in the diagnosis and management of idiopathic inflammatory myopathies. Rheum Dis Clin North Am 2002; 28: 859–90, viii. [PubMed: 12506776]
21. MAMMEN AL, CHUNG T, CHRISTOPHERSTINE L et al. : Autoantibodies against 3-hydroxy-3-methylglutaryl-coenzyme A reductase in patients with statin-associated autoimmune myopathy. Arthritis Rheum 2011; 63: 713–21. [PubMed: 21360500]
22. PINAL-FERNANDEZ I, CASAL-DOMINGUEZ M, HUAPAYA JA et al. : A longitudinal cohort study of the anti-synthetase syndrome: increased severity of interstitial lung disease in black patients and patients with anti-PL7 and anti-PL12 autoantibodies. Rheumatology (Oxford) 2017; 56: 999–1007. [PubMed: 28339994]
23. BOHAN A, PETER JB: Polymyositis and dermatomyositis (second of two parts). N Engl J Med 1975; 292: 403–7. [PubMed: 1089199]
24. GRIGGS RC, ASKANAS V, DIMAURO S et al. : Inclusion body myositis and myopathies. Ann Neurol 1995; 38: 705–13. [PubMed: 7486861]
25. RIDER LG, WERTH VP, HUBER AM et al. : Measures of adult and juvenile dermatomyositis, polymyositis, and inclusion body myositis: Physician and Patient/Parent Global Activity, Manual Muscle Testing (MMT), Health Assessment Questionnaire (HAQ)/Childhood Health Assessment Questionnaire (C-HAQ), Childhood Myositis Assessment Scale (CMAS), Myositis Disease Activity Assessment Tool (MDAAT), Disease Activity Score (DAS), Short Form 36 (SF-36), Child Health Questionnaire (CHQ), physician global damage, Myositis Damage Index (MDI), Quantitative Muscle Testing (QMT), Myositis Functional Index-2 (FI-2), Myositis Activities Profile (MAP), Inclusion Body Myositis Functional Rating Scale (IBMFRS), Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI), Cutaneous Assessment Tool (CAT), Dermatomyositis Skin Severity Index (DSSI), Skindex, and Dermatology Life Quality Index (DLQI). Arthritis Care Res (Hoboken) 2011; 63 (Suppl. 11): S118–57. [PubMed: 22588740]
26. TRAVIS WD, COSTABEL U, HANSELL DM et al. : An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med 2013; 188: 733–48. [PubMed: 24032382]
27. TAN EM, FELTKAMP TE, SMOLEN JS et al. : Range of antinuclear antibodies in “healthy” individuals. Arthritis Rheum 1997; 40: 1601–11. [PubMed: 9324014]
28. SATOH M, CHAN EK, HO LA et al. : Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. Arthritis Rheum 2012; 64: 2319–27. [PubMed: 22237992]
29. MARIN GG, CARDIEL MH, CORNEJO H, VIVEROS ME: Prevalence of antinuclear antibodies in 3 groups of healthy individuals: blood donors, hospital personnel, and relatives of patients with autoimmune diseases. J Clin Rheumatol 2009; 15: 325–9. [PubMed: 20009966]
30. MARIZ HA, SATO EI, BARBOSA SH, RODRIGUES SH, DELLAVANCE A, ANDRADE LE: Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear

antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. Arthritis Rheum 2011; 63: 191–200. [PubMed: 20954189]

31. FRITZLER MJ, PAULS JD, KINSELLA TD, BOWEN TJ: Antinuclear, anticytoplasmic, and anti-Sjögren's syndrome antigen A (SS-A/Ro) antibodies in female blood donors. Clin Immunol Immunopathol 1985; 36: 120–8. [PubMed: 3891174]
32. WATANABE A, KODERA M, SUGIURA K et al. : Anti-DFS70 antibodies in 597 healthy hospital workers. Arthritis Rheum 2004; 50: 892–900. [PubMed: 15022332]
33. FERNANDEZ SA, LOBO AZ, OLIVEIRA ZN, FUKUMORI LM, PÉRIGO AM, RIVITTI EA: Prevalence of antinuclear autoantibodies in the serum of normal blood donors. Rev Hosp Clín Fac Med Sao Paulo 2003; 58: 315–31. [PubMed: 14762490]
34. DELLAVANCE A, DE MELO CRUVINEL W, CARVALHO FRANCESCANTONIO PL et al. : Variability in the recognition of distinctive immunofluorescence patterns in different brands of HEp-2 cell slides. J Bras Patol Med Lab 2013; 49: 182–90.

Table I.

Indirect immunofluorescence prevalence and intensity in the different myositis subsets compared to the healthy comparators.

	HC (n=100)	DM (n=71)	IBM (n=53)	PM (n=69)
ANA positive	35% (35)	66% (47) ***	49% (26)	64% (44) ***
Weak	57% (20)	21% (10) ***	46% (12)	48% (21)
Moderate	17% (6)	38% (18) *	31% (8)	27% (12)
Intense	26% (9)	40% (19)	23% (6)	25% (11)
Moderate or intense	43% (15)	79% (37) ***	54% (14)	52% (23)

* $p<0.05$,

** $p<0.01$,

*** $p<0.001$.

Dichotomous variables were shown as %(n) and compared using the Chi-squared or Fisher's exact tests, as appropriate. Each group was compared with the healthy comparators.

HC: healthy comparator; DM: dermatomyositis; IBM: inclusion body myositis; PM: polymyositis.

Table II.

Indirect immunofluorescence pattern in the different myositis subsets compared to the healthy comparators.

	HC (n=35)	DM (n=47)	IBM (n=26)	PM (n=44)
Nuclear	89% (31)	91% (42)	69% (18)	77% (34)
Cytoplasmic	18% (6)	14% (6)	40% (10)	33% (14)
Mitotic	0% (0)	0% (0)	10% (2)	5% (2)

* $p<0.05$,

** $p<0.01$,

*** $p<0.001$.

Dichotomous variables were shown as %(n) and compared using the Chi-squared or Fisher's exact tests, as appropriate. Each group was compared with the healthy comparators.

HC: healthy comparator; DM: dermatomyositis; IBM: inclusion body myositis; PM: polymyositis.

Table III.

Nuclear indirect immunofluorescence pattern in the different myositis subsets compared to the healthy comparators.

	HC (n=31)	DM (n=42)	IBM (n=18)	PM (n=34)
Fine speckled	39% (12) *	64% (27) *	61% (11)	56% (19)
Homogeneous	29% (9) **	10% (4) *	6% (1)	0% (0) ***
Nuclear large/coarse speckled	13% (4)	10% (4)	6% (1)	3% (1)
Centromere	6% (2)	0% (0)	0% (0)	6% (2)
Nucleolar homogeneous	6% (2)	7% (3)	6% (1)	18% (6)
Nucleolar clumpy	3% (1)	2% (1)	6% (1)	6% (2)
Topo I speckled	3% (1)	0% (0)	0% (0)	0% (0)
Nuclear envelope punctate	0% (0)	2% (1)	11% (2)	6% (2)
Nuclear envelope smooth	0% (0)	0% (0)	6% (1)	0% (0)
Dense fine speckled	0% (0)	2% (1)	0% (0)	0% (0)
Discrete nuclear dots multiple	0% (0)	2% (1)	0% (0)	3% (1)
Nucleolar envelope punctate	0% (0)	0% (0)	0% (0)	3% (1)

* $p<0.05$,

** $p<0.01$,

*** $p<0.001$.

Dichotomous variables were shown as %(n) and compared using the Chi-squared or Fisher's exact tests, as appropriate. Each group was compared with the healthy comparators.

HC: healthy comparator; DM: dermatomyositis; IBM: inclusion body myositis; PM: polymyositis.

Table IV.

Comparison of clinical features during follow-up in IIFA-positive *vs.* IIFA-negative dermatomyositis patients.

	IIFA+ (n=47)	IIFA− (n=24)	*p*-value	Total (n=71)
Muscle weakness	91% (43)	92% (22)	1.0	92% (65)
Myalgia	15% (7)	21% (5)	0.5	17% (12)
DM-specific skin involvement	98% (46)	96% (23)	1.0	97% (69)
Sclerodactyly	0% (0)	0% (0)	1.0	0% (0)
Raynaud's phenomenon	43% (20)	50% (12)	0.6	45% (32)
Telangectasias	2% (1)	4% (1)	1.0	3% (2)
Ulcers	0% (0)	0% (0)	1.0	0% (0)
Carpal tunnel	2% (1)	0% (0)	1.0	1% (1)
Livedo reticularis	2% (1)	4% (1)	1.0	3% (2)
Mechanics hands	26% (12)	21% (5)	0.7	24% (17)
Calcinosis	9% (4)	12% (3)	0.7	10% (7)
Subcutaneous oedema	34% (16)	21% (5)	0.2	30% (21)
Puffy hands	0% (0)	8% (2)	0.1	3% (2)
Interstitial lung disease	19% (9)	21% (5)	1.0	20% (14)
Pulmonary hypertension	0% (0)	0% (0)	1.0	0% (0)
Dyspnea	62% (29)	58% (14)	0.8	61% (43)
Cough	21% (10)	21% (5)	1.0	21% (15)
Gastroesophageal reflux disease	45% (21)	54% (13)	0.4	48% (34)
Dysphagia	51% (24)	54% (13)	0.8	52% (37)
Arthritis	21% (10)	29% (7)	0.5	24% (17)
Arthralgia	70% (33)	46% (11)	0.05	62% (44)
Fever	11% (5)	17% (4)	0.5	13% (9)
Sicca syndrome	4% (2)	0% (0)	0.5	3% (2)
Pericarditis	0% (0)	0% (0)	1.0	0% (0)
Glomerulonephritis	0% (0)	0% (0)	1.0	0% (0)

* $p<0.05$,

** $p<0.01$,

*** $p<0.001$

Dichotomous variables are shown as %(n) and compared using the Chi-squared or Fisher's exact tests, as appropriate. IIFA: indirect immunofluorescence.

Table V.

Comparison of muscle strength and muscle enzyme levels in IIFA-positive *vs.* IIFA-negative dermatomyositis patients.

	IIFA+ (n=47)	IIFA− (n=24)	*p*-value	Total (n=71)
Mean hip flexor strength	*8.7 (1.6)*	*8.9 (1.9)*	*0.6*	*8.8 (1.7)*
Hip flexors strength at last visit	*8.7 (1.6)*	*9.0 (1.5)*	*0.6*	*8.8 (1.5)*
Mean arm abductor strength	*9.3 (1.0)*	*9.4 (1.2)*	*0.8*	*9.3 (1.1)*
Arm abductors strength at last visit	*9.2 (1.3)*	*8.8 (2.4)*	*0.5*	*9.0 (1.7)*
Median CK	*109 (58–280)*	*135 (55–204)*	*0.8*	*110 (58–280)*
Maximum CK	*155 (81–813)*	*168 (77–395)*	*0.6*	*155 (77–758)*
Mean aldolase	*8.8 (4.6)*	*7.1 (2.5)*	*0.1*	*8.2 (4.0)*
Maximum aldolase	*12.2 (12.3)*	*7.8 (2.9)*	*0.1*	*10.6 (10.1)*

* $p<0.05$,

** $p<0.01$,

*** $p<0.001$.

Strength values were expressed as means (SD) and CK as medians (Q1-Q3). Bivariate comparisons were made using Student's t-test for the strength and Wilcoxon rank-sum test for CK. IIFA: indirect immunofluorescence.