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Expression of CD163 and major histocompatibility complex class I as diagnostic markers for idiopathic inflammatory myopathies

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Abstract

Background To develop an inflammation-related immunohistochemistry marker-based algorithm that confers higher diagnostic ability for idiopathic inflammatory myopathies (IIMs) than IIM-related histopathologic features.

Methods Muscle biopsy tissues from 129 IIM patients who met the 2017 EULAR/ACR criteria and 73 control tissues from patients with non-inflammatory myopathies or healthy muscle specimens were evaluated for histological features and immunostaining results of CD3, CD4, CD8, CD20, CD68, CD163, MX1, MHC class I, MHC class II, and HLA-DR. Diagnostic algorithms for IIM were developed based on the results of the classification and regression tree (CART) analysis, which used immunostaining results as predictor variables for classifying patients with IIMs.

Results In the analysis set (IIM, $n=129$; control, $n=73$), IIM-related histopathologic features had a diagnostic accuracy of 87.6% (sensitivity 80.6%; specificity 100.0%) for IIMs. Notably, muscular expression of CD163 (99.2% vs. 20.8%, $p<0.001$) and MHC class I (87.6% vs. 23.1%, $p<0.001$) was significantly higher in the IIM group than in controls. Based on the CART analysis results, we developed an algorithm combining CD163 and MHC class I expression that conferred a diagnostic accuracy of 95.5% (sensitivity 96.1%; specificity 94.5%). In addition, our algorithm was able to correctly diagnose IIM in 94.1% (16/17) of patients who did not meet the 2017 EUALR/ACR criteria but were diagnosed as having IIMs by an expert physician.

Conclusions Combination of CD163 and MHC class I muscular expression may be useful in diagnosing IIMs.

Keywords Myositis, CD163 antigen, Histocompatibility antigens class I, Immunohistochemistry

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Introduction

Idiopathic inflammatory myopathies (IIMs) are a group of heterogeneous muscle disorders with clinical characteristics of weakening of the skeletal muscle, decreased muscle endurance, and muscle fatigue. IIMs have characteristic histopathologic features related to inflammation such as the presence of mononuclear inflammatory cell infiltrates in muscle tissues [1–3], which serve as the diagnostic criterion for IIMs [4]. However, IIM diagnosis remains a challenge, notably considering muscle biopsy analysis. Histopathologic examination solely based on hematoxylin and eosin (H&E) staining often fails to show the typical inflammatory features of IIMs in muscle tissues in 20.1–62.5% of patients with IIMs [5–8]. Ruling out the non-inflammatory myopathy diagnosis is crucial as treatment and care are very different. Therefore, there is a need to improve the method of evidence-based identification of muscle inflammation in the diagnosis of IIMs.

In order to develop methods for evidence-based identification of muscle inflammation for the diagnosis of IIMs, previous studies investigated the muscular expression of immunohistochemistry (IHC) markers for inflammation associated with IIMs. Specifically, MHC class I and MHC class II expression on muscle specimens are able to distinguish IIMs from non-IIMs with high sensitivity (MHC class I, 78–100%; MHC class II, 60.5–93%) and specificity (MHC class I, 7.1–95%, MHC class II, 90.8–100%) [7, 9–11]. However, MHC class I has a low specificity and MHC class II has a low sensitivity and thus has limited applicability in clinical practice. Various inflammatory markers including BAFF receptor, MX-1, CD3, CD4, CD8, CD19, CD68, and CD163 were also shown to be useful markers for the diagnosis of IIMs [12–18]. However, as most of these studies were small-scale studies and utilized only a small number of inflammatory markers, they could not establish optimal inflammatory markers and their combination for improving the accuracy of IIM diagnosis. As a result, diagnosis using muscular expression of inflammatory markers is included in the routine stains for all new biopsies in the Recommended Standards for Muscle Pathology of The EURO-NMD pathology working group [19] but is not included in the 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies (2017 EULAR/ACR criteria for IIMs) [20].

We hypothesized that certain inflammation-related IHC markers and their combinations could be used to better distinguish between IIMs and non-IIMs than histopathologic examination based on H&E staining alone. Accordingly, we investigated and compared the expression of various inflammatory markers on muscle biopsy specimens from patients with IIMs that met the 2017 EULAR/ACR criteria for IIMs. In addition, we sought to develop an optimized combination of inflammatory markers that can distinguish between patients with IIMs and controls.

Materials and methods

Patients

The study population consisted of a retrospective cohort of 146 patients with IIMs diagnosed by an expert neurologist or rheumatologist at a tertiary hospital between January 2001 and March 2017 who had available muscle biopsy specimens that were obtained during diagnostic evaluation. Of them, 129 patients met the 2017 EULAR/ACR criteria for IIMs [20] (Supplementary Figure S1) and were included in the study. The patient charts were retrospectively reviewed to collect data on demographic features, clinical characteristics at disease onset, serum levels of muscle enzymes (i.e., creatine phosphokinase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and myoglobin), erythrocyte sedimentation rate, C-reactive protein level, and autoantibody status at the time of diagnosis. The control group consisted of a total of 73 muscle biopsy specimens, including 57 myopathy muscles from patients with non-IIM who had undergone muscle biopsies during diagnostic evaluation and 16 normal muscles from patients who had undergone other surgeries (Supplementary Table S1).

For validation of our proposed histopathologic algorithms, we additionally used the muscle biopsy specimens of 68 IIM patients who met the 2017 criteria between January 2019 and December 2020. In this process, 10 non-IIM muscle disease cases and 4 normal muscles from patients who had undergone other surgeries were used as controls. The present study protocol was approved by the Institutional Review Board of Asan Medical Center (approval #2021−0881; approval date: October 10, 2021).

Histopathologic analysis

Histopathologic features of muscle biopsy specimens were evaluated using H&E staining. Inflammatory myositis-like features, including fiber size variation and atrophic fibers, fiber necrosis, internal nuclei, moth-eaten fibers, core-like area, fiber splitting, and perifascicular atrophy were reviewed by a neuropathologist (S. J. Nam). The severity of histopathologic features was categorized as negative, mild, moderate, and severe. Cases were classified as having histopathological features consistent with IIM if histopathological features specific to IIM or T-cell infiltration (i.e., extensive or sheet-like) were found. To analyze the importance of IHC in histopathological examination, we defined cases that exhibited sufficient characteristics for diagnosing IIMs based on H&E

staining alone, without the use of IHC markers, as having "IIM-related histopathologic features".

To perform a semiquantitative analysis of IHC markers, a systematic review for grading and patterning was implemented. The patterns of immune cell markers including CD3, CD4, CD8, CD20, CD68, and CD163 were categorized into endomysial, perivascular and perimysial, and both perivascular/perimysial and endomysial patterns. MHC molecules and MX1 were separately evaluated in immune cells, vascular endothelial cells, and myofibers. The results of IHC staining were analyzed semi-quantitatively, and we defined focal expression (less than 5%) as mild, partial expression (5–50%) as moderate, and diffuse expression (more than 50%) as strong. Additionally, the pathological assessment was conducted by a single neuropathologist who was blinded to the clinical data and final diagnosis to ensure unbiased evaluation of the samples.

Immunohistochemical staining

Whole Sect. (4-μm-thick) of representative formalin-fixed paraffin-embedded tissue blocks of muscle specimens were used for IHC analysis using T cell markers (CD3, CD4, and CD8), B cell marker (CD20), macrophage marker (CD68), M2 macrophage marker (CD163), systemic inflammatory marker associated with type 1 interferon (MX1) [21, 22], MHC class I marker (HLA-ABC), and MHC Class II marker (HLA-DR+DP+DQ).

BenchMark XT Autostainer (Ventana Medical Systems, Inc., Tucson, AZ, USA) was used for immunostaining using antibodies against CD3 (1:100, Rabbit monoclonal, clone POLY, catalog No.A0452, DAKO, Denmark, Glostrup), CD4 (1:16, Rabbit monoclonal, clone SP35, catalog No.790–4423, VENTANA, Tusan, USA), CD8 (1:400, Mouse monoclonal, clone C8/144B, catalog No.M7103, CELL MARQUE, CA, USA), CD20 (1:400, Mouse monoclonal, clone L26, catalog No.M0755, DAKO, Denmark, Glostrup), CD68 (1:2000, Mouse monoclonal, clone KP1, catalog No.M0814, DAKO, Denmark, Glostrup), CD163 (1:400, Mouse monoclonal, clone MRQ-26, catalog No.163 M-16, CELL MARQUE, CA, USA), MX1 (1:500, Rabbit polyclonal, clone N2C2, catalog No. GTX110256, GENETEX, CA, USA), HLA Class I ABC (1:10000, clone EMR8-5, catalog No. ab70328, Abcam, Inc., Cambridge, UK), HLA-DR+DP+DQ (1:2500, clone CR3/43, catalog No. ab7856, Abcam, Inc., Cambridge, UK), and HLA-DR (1:5000, clone TAL 1B5, catalog No. ab20181, Abcam, Inc., Cambridge, UK). In the validation set, immunostaining was performed only for CD163 and MHC class I. Secondary antibodies were detected using the OptiView DAB IHC Detection Kit (Roche Tissue Diagnostics). A negative control was not used.

Statistical analysis

We performed the classification and regression tree (CART) analysis [23] to classify IIM patients using histopathologic features and IHC results (e.g., CD3, CD4, CD8, CD20, CD68, CD163, MX1, MHC class I, MHC class II, HLA-DR) as predictor variables using the R package rpart (REF: Accessible online: https://cran.r-project.org/web/packages/rpart/rpart.pdf). Then, we constructed more practical trees with the most powerful variable selected from the primitive model, combining other various histopathologic features and IHC results. These analyses were conducted respectively by including and excluding (due to the complexity of the test) histopathologic features suitable for IIM on H&E staining. The predictive capacity of each combination was calculated. In addition, this combination was applied to patients who were diagnosed with IIMs by the attending physician but did not meet the 2017 EULAR/ACR criteria for IIMs. Mann–Whitney *U* test was performed for intergroup comparisons. Results are presented as mean±standard error of the mean (SEM). P values <0.05 were considered statistically significant.

Results

Clinical characteristics of patients with IIMs

The clinical characteristics of the 129 study patients are summarized in Supplementary Table S2. Patients with IIMs (66 patients with dermatomyositis [DM] and 63 patients with polymyositis [PM]) were followed up until January 2018 (follow-up duration: mean, 5.7±5.0 years; median, 3.4 years [interquartile range (IQR), 1.1–6.2]). Approximately two-thirds (65.9%) of the patients were female, and the mean age at diagnosis of IIM was 51.5±14.8 years. Of the patients, 27 (20.9%) were diagnosed with a malignancy before or after the diagnosis of IIM. Interstitial lung disease was diagnosed in 49 (38.0%) patients at the time of diagnosis of IIM, and 37 patients (28.7%) died during the follow-up period.

Among patients with IIMs, those with PM had significantly higher levels of CRP (2.8±4.4 mg/dL vs. 1.5±2.5 mg/dL, $p=0.041$), alanine aminotransferase (172±173 IU/L vs. 92±110 IU/L, $p=0.003$), creatine phosphokinase (5590±5987 U/L vs. 3361±5468 U/L, $p=0.029$), and myoglobin (2434±3596 ng/mL vs. 921±1461 ng/mL, $p=0.005$) than those with DM. In contrast, ANA titer was significantly higher in DM patients (221±375 vs. 92±304, $p=0.046$).

Histopathological characteristics of patients with IIMs

Histopathologic features consistent with IIM were observed in 104 patients (80.6%) (Table 1). Fiber size variation and atrophic fibers were common in patients with IIM (94.6%). Perifascicular atrophy, perimysial lymphocytic infiltration, and endomysial lymphocytic

Table 1 Histopathologic findings in patients with idiopathic inflammatory myopathies and controls

	Patients with IIMs (*N* = 129)	Control (*N* = 73)		*P* value	Dermatomyositis (*N* = 66)	Polymyositis (*N* = 63)	*P* value
		Non-Myopathy† (*N* = 16)	Patients with non-IIM‡ (*N* = 57)				
Histopathologic features of IIMs	104 (80.6%)	N/A	N/A	-	46 (69.7%)	58 (92.1%)	0.003
Fiber size variation and atrophic fibers	122 (94.6%)	N/A	N/A	-	60 (90.9%)	62 (98.4%)	0.12
Fiber necrosis	68 (52.7%)	N/A	N/A	-	24 (36.4%)	44 (69.8%)	< 0.001
Internal nuclei	71 (55.0%)	N/A	N/A	-	25 (37.9%)	46 (73.0%)	< 0.001
Moth eaten fibers	6 (4.7%)	N/A	N/A	-	2 (3.0%)	4 (6.3%)	0.43
Core-like area	0 (0.0%)	N/A	N/A	-	0 (0.0%)	0 (0.0%)	-
Fiber splitting	29 (22.5%)	N/A	N/A	-	11 (16.7%)	18 (28.6%)	0.16
Perifascicular atrophy	23 (17.8%)	N/A	N/A	-	8 (12.1%)	15 (23.8%)	0.13
Endomysial lymphocytic infiltration	86 (66.7%)	N/A	N/A	-	36 (54.5%)	50 (79.4%)	0.005
Perimysial lymphocytic infiltration	91 (70.5%)	N/A	N/A	-	49 (74.2%)	42 (66.7%)	0.45
Presence of $CD3^+$ cells	118 (91.5%)	9 (56.2%)	26 (53.1%)	< 0.001	57 (86.4%)	61 (96.8%)	0.07
Presence of $CD4^+$ cells	87 (67.4%)	4 (25.0%)	4 (7.4%)	< 0.001	40 (60.6%)	47 (74.6%)	0.13
Presence of $CD8^+$ cells	91 (70.5%)	9 (56.2%)	15 (27.8%)	< 0.001	39 (59.1%)	52 (82.5%)	0.006
Presence of $CD20^+$ cells	60 (46.5%)	0 (0.0%)	0 (0.0%)	< 0.001	26 (39.4%)	34 (54.0%)	0.14
Presence of $CD68^+$ cells	123 (95.3%)	0 (0.0%)	16 (30.2%)	< 0.001	61 (92.4%)	62 (98.4%)	0.21
Presence of $CD163^+$ cells	128 (99.2%)	0 (0.0%)	15 (26.8%)	< 0.001	66 (100.0%)	62 (98.4%)	0.49
MX1 expression on myofibers*	51 (39.5%)	1 (6.2%)	26 (53.1%)	0.004	41 (62.1%)	10 (15.9%)	< 0.001
MX1 expression on capillaries*	119 (92.2%)	11 (68.8%)	43 (87.8%)	0.019	62 (93.9%)	57 (90.5%)	0.52
MX1 expression on immune cells*	87 (67.4%)	8 (50.0%)	21 (42.9%)	0.008	51 (77.3%)	36 (57.1%)	0.024
MHC class I expression on myofibers*	113 (87.6%)	5 (31.2%)	10 (20.4%)	< 0.001	10 (15.2%)	7 (11.1%)	0.68
MHC class I expression on capillaries*	129 (100.0%)	15 (93.8%)	45 (91.8%)	0.004	41 (62.1%)	45 (71.4%)	0.35
MHC class I expression on immune cells*	127 (98.4%)	4 (25.0%)	19 (38.8%)	< 0.001	42 (63.6%)	53 (84.1%)	0.015
MHC class II expression on myofibers*	17 (13.2%)	2 (12.5%)	16 (32.7%)	0.011	63 (95.5%)	50 (79.4%)	0.012
MHC class II expression on capillaries*	86 (66.7%)	4 (25.0%)	31 (63.3%)	0.005	66 (100.0%)	63 (100.0%)	1.00
MHC class II expression on immune cells*	95 (73.6%)	7 (43.8%)	22 (44.9%)	< 0.001	64 (97.0%)	63 (100.0%)	0.50
HLA-DR expression on myofibers*	90 (69.8%)	2 (12.5%)	10 (20.4%)	< 0.001	46 (69.7%)	44 (69.8%)	1.00
HLA-DR expression on capillaries*	128 (99.2%)	15 (93.8%)	48 (98.0%)	0.134	65 (98.5%)	63 (100.0%)	1.00
HLA-DR expression on immune cells*	126 (97.7%)	9 (56.2%)	33 (67.3%)	< 0.001	64 (97.0%)	62 (98.4%)	1.00

HLA = Human leukocyte antigen; IIMs = idiopathic inflammatory myopathies; MHC = Major histocompatibility complex; MX1 = Myxovirus resistance protein 1; N/A = not available

†Non-Myopathy: Normal muscles from patients who had undergone other surgeries

‡Patients with non-IIM: Myopathy muscles from patients with non-IIM who had undergone muscle biopsies during diagnostic evaluation

*Staining was performed in 65 cases of the control group due to missing values

infiltration, which are histopathological features specific to IIM, were observed in 23 (17.8%), 91 (70.5%) and 86 (66.7%) patients, respectively. Fiber splitting and moth-eaten fibers were observed in 29 (22.5%) and 6 (4.7%) patients, respectively.

Histopathological features of patients with DM and PM are summarized in Table 1. Histopathologic features consistent with IIM were more common in the PM group than in the DM group (92.1% vs. 69.7%; $p = 0.003$). Likewise, fiber necrosis (69.8% vs. 36.4%; $p < 0.001$) and internal nuclei (73.0% vs. 37.9%; $p < 0.001$) were more common in the PM group. Moth-eaten fibers (6.3% vs. 3.0%; $p = 0.43$) and fiber splitting (28.6% vs. 16.7%; $p = 0.16$) were more common in the PM group as well, albeit without statistical significance. Endomysial lymphocytic infiltration, which is a specific histopathologic feature of PM, was significantly higher in the PM group than in the DM group (79.4% vs. 54.5%; $p = 0.005$). In contrast, histopathologic features specific to DM including perimysial lymphocytic infiltration (66.7% vs. 74.2%; $p = 0.45$) and perifascicular atrophy (23.8% and 12.1%; $p = 0.13$) did not show a significant difference between the PM and DM groups.

Expression of immune cells and inflammation-associated markers

To assess histopathologic changes of immune cell infiltration and inflammation-associated markers in patients with IIMs, we performed IHC stainings for immune-related markers including CD3, CD4, CD8, CD20, CD68, CD163, MX1, MHC class I, MHC class II, and HLA-DR on muscle biopsy specimens (Table 2; Fig. 1). To compare

Table 2 Sensitivity, specificity, and predictive values of various inflammatory markers and proposed algorithms for the histopathologic prediction of idiopathic inflammatory myopathies

	Analysis set				
	Sensitivity	**Specificity**	**PPV**	**NPV**	**Accuracy**
Histopathologic features of IIMs	80.6%	100.0%	100.0%	74.5%	87.6%
Presence of $CD3^+$ cells	91.5%	51.4%	77.6%	76.6%	76.3%
Presence of $CD163^+$ cells	99.2%	79.2%	89.5%	98.3%	92.1%
Presence of $CD163^+$ cells in endomysial or endomysial/perivascular area	84.5%	97.3%	98.2%	78.0%	89.1%
MHC class I on myofibers	87.6%	76.9%	88.3%	75.8%	84.0%
HLA-DR on myofibers	69.8%	81.5%	88.2%	57.6%	73.7%
MHC class II on myofibers	13.2%	72.3%	45.6%	29.6%	33.5%
Proposed algorithm 1[†]	96.1%	94.5%	96.9%	93.2%	95.5%
Proposed algorithm 2[‡]	97.7%	94.5%	96.9%	95.8%	96.5%
	Validation set				
	Sensitivity	**Specificity**	**PPV**	**NPV**	**Accuracy**
Histopathologic features of IIMs	63.2%	100.0%	100.0%	35.9%	69.5%
Presence of $CD163^+$ cells	94.1%	85.7%	97.0%	75.0%	92.7%
Presence of $CD163^+$ cells in endomysial or endomysial/perivascular area	67.6%	100.0%	100.0%	38.9%	73.2%
MHC class I on myofibers	95.6%	100.0%	100.0%	82.4%	96.3%
Proposed algorithm 1[†]	94.1%	100%	100%	77.8%	95.1%
Proposed algorithm 2[‡]	94.1%	100%	100%	77.8%	95.1%

[†]Combination of CD163 pattern and MHC class I on myofibers

[‡] Combination of histopathologic examination, CD163 pattern, and MHC class I on myofibers

the expression of inflammation-related markers in tissues with other myopathies and normal muscle, a control group consisting of 73 muscle biopsy specimens was constructed and the same set of IHC stainings was performed.

All immune cell markers showed significantly higher expression in the IIM group than in the control group, including CD3 (91.5% vs. 53.8%, $p<0.001$), CD4 (67.4% vs. 11.4%, $p<0.001$), CD8 (70.5% vs. 34.3%, $p<0.001$), CD20 (46.5% vs. 0.0%, $p<0.001$), CD68 (95.3% vs. 23.2%, $p<0.001$) and CD163 (99.2% vs. 20.8%, $p<0.001$). $CD20^+$ B-cell clusters were only observed in the IIM group, whereas other inflammatory cells were also present in the control group. Perivascular/perimysial patterns tended to be more common in the IIM group than in the control group, especially in terms of CD3, CD4, and CD8 (Fig. 2). On the other hand, the endomysial pattern of macrophage markers including CD68 and CD163 was more common in the IIM group than in the control group.

Expression of inflammatory markers in patients with DM and PM are summarized in Table 1. Of the various markers, only the expression of CD8 (82.5% vs. 59.1%, $p=0.006$) was significantly higher in the PM group compared with the DM group, and the two groups did not show significant differences in terms of the expression of other immune cell markers. Regarding the patterns of immune cell infiltration in terms of CD3 and CD8, the DM group showed a significant perivascular/perimysial pattern and the PM group showed a significant endomysial pattern ($p<0.001$ for both, Fig. 2). The patterns of $CD4^+$ cells, $CD20^+$ B-cells, and macrophage markers including CD68 and CD163 did not significantly differ between the DM and PM groups.

Inflammation-associated markers, including MX1, MHC class I, MHC class II, and HLA-DR were stained for myofibers, capillaries, and immune cells, respectively. The IIM group showed a significantly higher frequency of MHC class I expression in myofibers (87.6% vs. 23.1%, $p<0.001$), capillaries (100.0% vs. 92.3%, $p=0.004$), and immune cells (98.4% vs. 35.4%, $p<0.001$) compared with the control group. Also, the IIM group showed a significantly higher frequency of HLA-DR expression on myofibers (69.8% vs. 18.5%, $p<0.001$) and HLA-DR on immune cells (97.7% vs. 64.6%, $p<0.001$). Expression of MX1 (67.4% vs. 44.6%, $p=0.004$) and MHC class II (73.6% vs. 44.6%, $p<0.001$) on immune cells was significantly more common in the IIM group as well.

When comparing the DM and PM groups, the expression of MX1 on myofibers (62.1% vs.15.9%, $p<0.001$), MX1 on immune cells (77.3% vs. 57.1%, $p=0.024$), and MHC class II on myofibers (95.5% vs. 79.4%, $p=0.012$) was significantly more common in the DM group than in the PM group. Expression of MHC class I on immune cells (63.6% vs. 84.1%, $p=0.015$) was significantly more common in the PM group than DM group (Table 1).

Combination of inflammatory markers and development of histopathologic algorithms for the prediction of IIMs

Using the decision tree generated by CART analysis, we combined different variables to differentiate between patients with IIMs who met the 2017 EULAR/ACR criteria for IIM and controls. In the first decision tree, the first

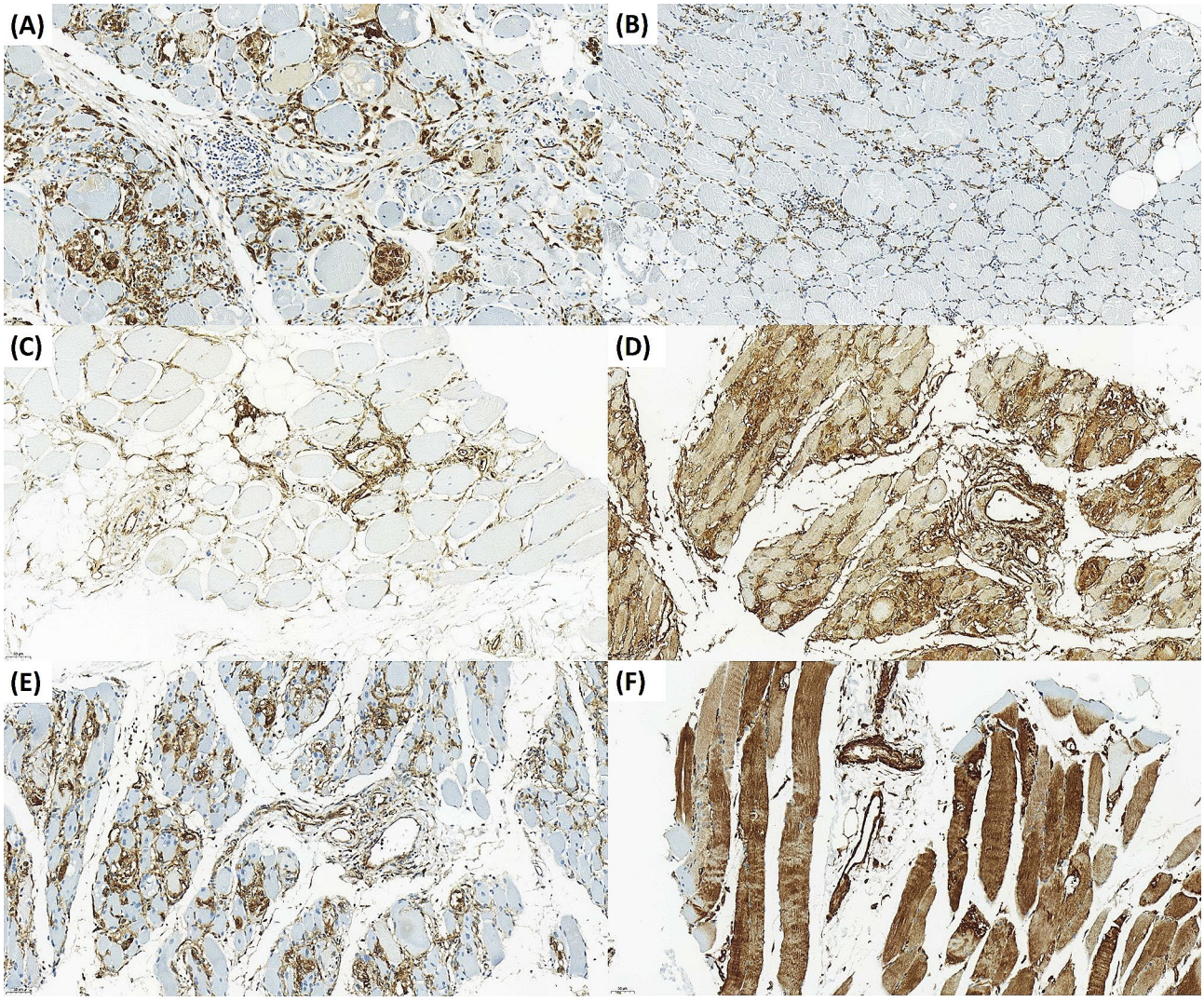

Fig. 1 CD163, MHC class I, and MX1 muscular expression in patients with idiopathic inflammatory myopathies. (**A**, **B**) CD163 expression on the endomysial area of a muscle biopsy specimen from a patient with polymyositis (×200); (**C**) MHC class I expression on immune cells and capillary in a muscle biopsy specimen from a patient with dermatomyositis (×200); (**D**) MHC class I expression on immune cells, capillary, and myofibers in a muscle biopsy specimen from a patient with dermatomyositis (×200); (**E**) MX1 expression on immune cells and capillary in a muscle biopsy specimen from a patient with dermatomyositis (×200); (**F**) MX1 expression on immune cells, capillary, and myofibers in a muscle biopsy specimen from a patient with dermatomyositis (×200)

variable selected was CD163 expression; the combination of CD163 expression, CD163 expression in the endomysial or the endomysial/perivascular areas, and MHC class I expression on myofibers provided a significant stratification between the IIM patients and controls. We generated another decision tree using IIM-related histopathological features as the first variable and combined it with CD163 and MHC class I expression. The combination of IIM-related histopathologic features, CD163 expression, CD163 expression in the endomysial or the endomysial/perivascular areas, and MHC class I expression on myofibers provided strong stratification between the IIM patients and controls. Based on the CART analysis results, we constructed two algorithms—algorithm 1 (CD163 expression+CD163 expression in the endomysial or the endomysial/perivascular areas+MHC class I expression on myofibers) and algorithm 2 (IIM-related histopathologic features+algorithm 1) —for the histopathologic prediction of IIM (Fig. 3).

Algorithms 1 and 2 both showed good classification performance in the analysis set (accuracy: 95.5% and 96.5%) and in the validation set (accuracy: 95.1% and 95.1%). These two algorithms showed higher sensitivity and negative predictive value compared with histopathologic examination only in both the analysis set and the validation set (Table 2). The algorithms showed better IIM classification performance than the use of histopathologic features alone or IHC markers such as MHC class I alone (Table 2). In addition, we applied these algorithms to 17 patients who were diagnosed by an expert physician

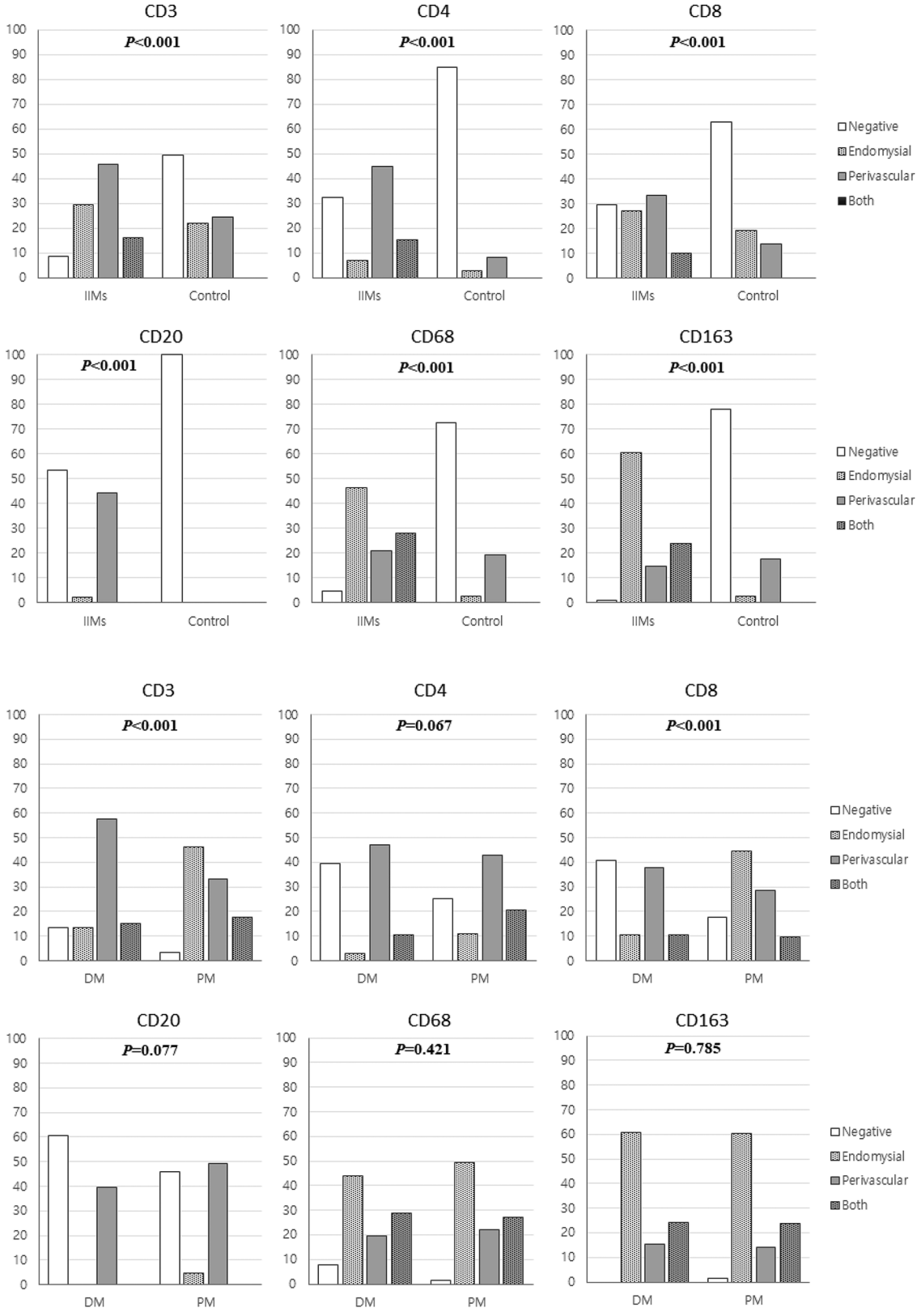

Fig. 2 Immune cell infiltration patterns in (**A**) patients with IIMs and control cases, and (**B**) in patients with dermatomyositis and polymyositis

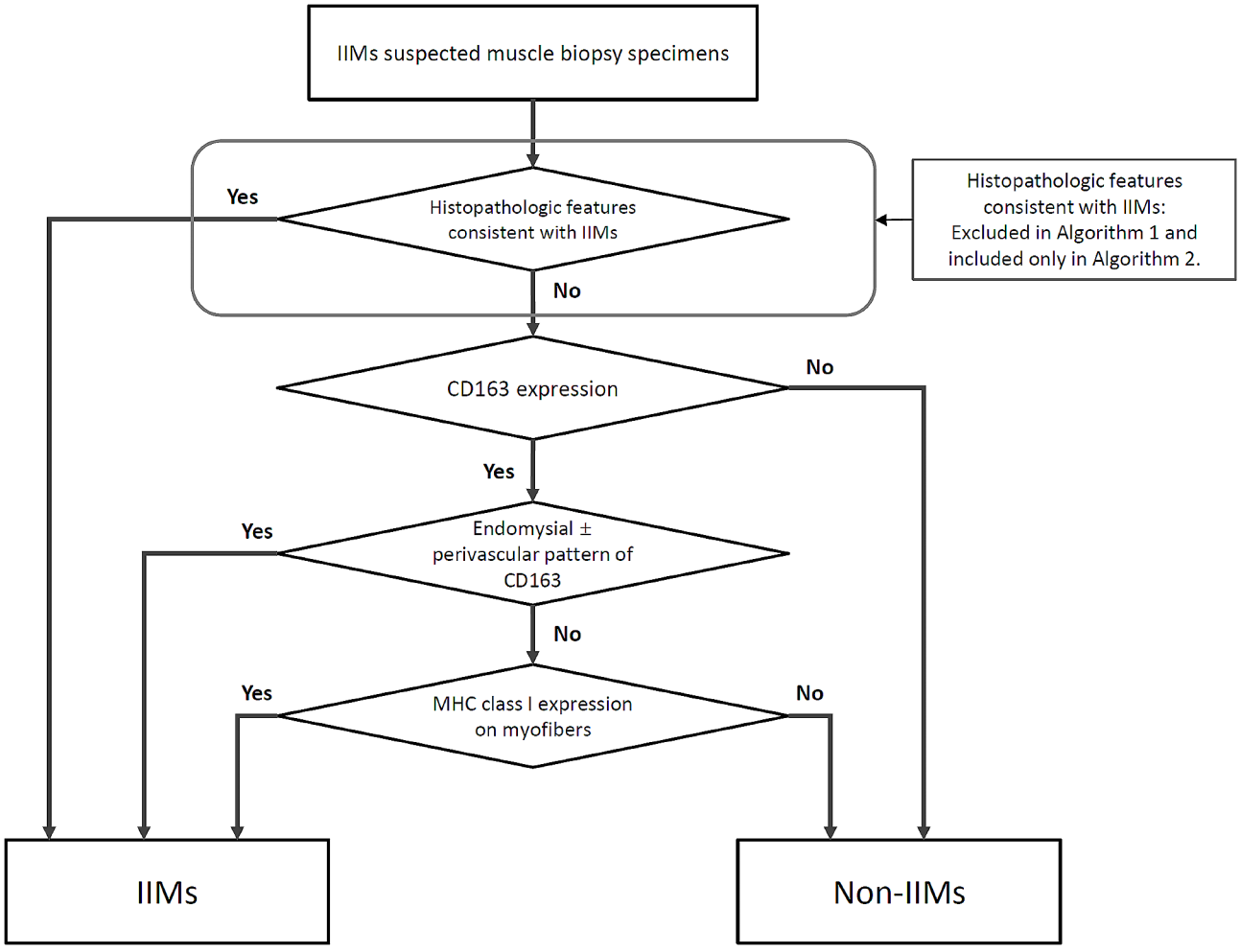

Fig. 3 Proposed histopathologic algorithms for the prediction of idiopathic inflammatory myopathies using the combination of CD163 and MHC class I expression. Histopathologic features consistent with IIMs were only included in algorithm 2

but did not meet the 2017 EUALR/ACR criteria for IIMs. We found that both algorithms were able to classify 16 of 17 (94.1%) patients as having IIMs (Supplementary Table S3). ROC analysis showed that the AUC for algorithm 1 and algorithm 2 was 0.953 (95% CI: 0.922–0.984) and 0.961 (95% CI: 0.932–0.99), respectively (Supplementary Fig. 2).

In addition to the combination of CD163 expression and MHC class I expression on myofibers, we also investigated the performance of other combinations for the stratification between IIM patients and controls (Supplementary Table S4). In this analysis, CD163 expression was selected as the first variable based on the results of the preceding CART analysis; for the second variable, we used CD3 expression, CD4 expression, CD20 expression, CD68 expression, MX1 expression on capillaries, MX1 expression on immune cells, MHC class I expression on capillaries, MHC class II expression on myofibers, or HLA-DR expression on immune cells. Although inferior to algorithms 1 and 2 described above, all of these algorithms had high accuracy (range, 91.6–94.8%).

Discussion

Identification of inflammation in the muscle tissue is important in the diagnosis of IIMs. However, many muscle biopsy specimens do not show histopathological findings specific to IIMs, including inflammatory cell infiltration [5–8]. In this study, almost one-fifth (19.4%; 25/129) of patients with IIMs who met the 2017 EULAR/ACR criteria showed almost normal or only non-specific changes under H&E staining. We therefore devised two algorithms that can improve the diagnostic accuracy of IIMs in muscle biopsy tissue specimens through the combination of inflammatory markers. The combination of CD163 and MHC class I expression in muscle biopsy specimens showed higher diagnostic accuracy compared with histopathologic examination or single marker staining alone. In addition, our algorithms were able to classify most (94%) of the patients who were diagnosed with IIMs

by expert physicians but did not meet the 2017 EULAR/ACR criteria as having IIMs.

In the clinic, our proposed algorithms may help the diagnosis of IIMs even in the absence of a muscle pathologist. In addition, algorithms that combine readouts for histopathological features of IIMs are cost-effective considering that IHC tests can only be performed on a limited number of samples. Furthermore, as our proposed algorithms were able to classify most of the patients who did not meet the 2017 EULAR/ACR criteria as having IIMs, they could be useful auxiliary diagnostic tools that can aid both clinically and pathologically in the diagnosis of IIMs. In addition, we found that the combinations of CD163 expression with other inflammatory markers such as CD3, CD4, CD68, MX1, MHC class II, and HLA-DR were able to distinguish IIM patients from controls with high accuracy of up to 94.8%. These results may serve as a guide for future studies investigating the potential use of inflammatory markers in the diagnosis of IIMs.

Several studies in IIMs reported that CD163 expression and MHC class I expression are associated with pathogenesis in addition to having a diagnostic significance. The activation and function of macrophages are significantly linked with the pathogenesis of a variety of autoimmune diseases. Peng et al. reported that the serum level of soluble CD163, which is mainly secreted by monocyte/macrophage lineage cells, was significantly higher in patients with PM and DM compared with healthy controls. [16] Inflammatory infiltrates containing $CD163^+$ macrophages are commonly observed in the diseased muscles in cases of PM and DM [15, 16], where activated macrophages directly induce inflammation and mediate inflammatory responses via lymphocyte activation [24, 25]. A previous study of 11 patients found a significantly higher number of $CD163^+$ macrophages in the biopsy specimens of both symptomatic and asymptomatic muscles than in those from controls [15]. Considering the existing evidence of CD163 as a marker of macrophage activation, including the present finding of CD163 expression in muscle tissue, our data highlight the role of macrophage activation in the pathogenesis of IIMs.

MHC class I expression on the sarcolemma, which is absent in normal muscle fibers [26, 27], is up-regulated in inflammatory myopathies [9, 26, 28]. MHC class I molecules are necessary for antigen-specific T cell-mediated cytotoxicity and can mediate the response against surface antigens on myofibers [29]. Previous studies have also shown that MHC class I can behave as a pathogenic molecule since its expression can precede lymphocytic cell infiltration, and transgenic mice overexpressing MHC class I develop a severe myopathy even in the absence of inflammation [30–33]. A recent study reported that MHC class I immunostaining was useful in distinguishing IIMs from non-inflammatory myopathies or neurogenic disorders (sensitivity of 0.95 and specificity of 0.82) [34]. The immunohistological detection of CD163 and MHC class I antigens in muscle biopsy tissues might serve as a diagnostic criterion for IIM.

The combination of CD163 and CD20 expression also had high sensitivity and specificity for the diagnosis of IIMs, albeit with lower accuracy than the combination of CD163 and MHC class I expression. In fact, CD20 had the highest specificity for diagnosing IIMs as there was no $CD20^+$ B cell infiltration in the control group including cases with various non-inflammatory myopathies (Table 1). B cells are known to be associated with autoantibodies and disease activity of myositis [35], and the maturation of B cells infiltrating myositis tissues into antibody-producing plasma cells has also been reported [36]. Therefore, it is possible that B cell infiltration in myositis is related to the severity and prognosis of myositis. Also, the presence of $CD20^+$ B cells may be useful in differentiating myositis from other myopathies.

This study has several limitations in addition to those associated with its retrospective design. First, as we could not obtain blood samples that correspond with the muscle biopsy specimens, we could not analyze the systemic levels of cytokines associated with various inflammatory markers expressed in muscle tissues. Also, among the different subtypes of IIMs, we could not include cases of inclusion body myositis and juvenile myositis because of their rarity in South Korea; moreover, as we classified the patients according to the 2017 EULAR/ACR criteria for IIMs, cases of immune-mediated necrotizing myopathies and anti-synthetase syndrome may have been categorized as PM, which could have contributed to the failure to establish an algorithm for distinguishing between DM and PM. In addition, since IBM patients were not included in this study, we do not know whether our proposed diagnostic algorithm is effective in IBM. Our study required a significant amount of muscle samples for IHC staining of various markers. IBM is rare in Korea due to ethnic differences, as a result, there were no additional available muscle samples from IBM patients. Additional studies are needed to expand the coverage of diagnostic algorithms to all subgroups of IIMs. Moreover, our study did not investigate myositis-specific autoantibodies other than anti-Jo1, which are essential for diagnosing and predicting the prognosis of IIMs. In the clinical setting in Korea, testing for myositis-specific autoantibodies is uncommon, and the absence of this data prevented us from presenting the precise subgroups and clinical information of IIMs. Therefore, while our study results may not significantly impact the subgrouping of IIMs, they do provide a highly accurate and objective method for distinguishing between IIMs that require long-term immunomodulatory treatment and non-inflammatory

myopathies. In addition, glucocorticoid administration was initiated at a median of 4.0 days (IQR, 2.0–11.5) before muscle biopsy in about half (52.7%) of the patients with IIMs, which may have led to some false negative results of histopathologic features and immunohistochemical staining. Also, the control group was heterogeneous (healthy muscles+various non-inflammatory myopathies) and had a rather small number of specimens; nevertheless, the validation set showed consistent results with the analysis set. Lastly, our proposed algorithms were not validated using an external cohort.

Conclusions

In this study, we found that muscle biopsy specimens from patients with IIMs showed distinct expression patterns of various inflammatory IHC markers compared with controls. Based on those results, we developed two algorithms based on an optimized combination of CD163 expression and MHC class I muscular expression, which showed significantly higher diagnostic accuracy compared with histopathologic examination based on H&E staining alone. Combined muscular expression of CD163 and MHC class I may be useful in diagnosing IIMs.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13075-024-03364-z.

Supplementary Material 1

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Author contributions

Soo Jeong Nam and Bin Yoo had full access to all the data in the study and take full responsibility for the integrity of the data and accuracy of the data analysis. Byeongzu Ghang, Soo Jeong Nam, and Bin Yoo conceptualized and designed the study. Byeongzu Ghang, So Hye Nam, Soo Jeong Nam, Wonho Choi, Doo-Ho Lim, Soo Min Ahn, Ji Seon Oh, Seokchan Hong, and Hwa Jung Kim acquired the data and performed the analyses and interpretation. Statistical analysis was performed by Byeongzu Ghang, Soo Jeong Nam, and Hwa Jung Kim. Byeongzu Ghang, Hwa Jung Kim, Soo Jeong Nam, and Bin Yoo drafted the manuscript. Soo Jeong Nam, Bin Yoo, Yong-Gil Kim, Chang-Keun Lee, and Jinseok Kim supervised the study. All authors critically reviewed the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval

The study protocol was approved by the Institutional Review Board of Asan Medical Center (approval #2021 – 0881; approval date: October 10, 2021).

Patient consent for publication

Not applicable.

Competing interests

Byeongzu Ghang is the CEO of InDream Healthcare Inc., a digital healthcare company that develops an automatic drug interaction searching program and app (https://www.medi-chart.com). Byeongzu Ghang, Bin Yoo, and Soo Jeong Nam, along with their affiliated institutions, hold patents related to this research that are restricted to Korea.

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