Immunopathological features of myopathy associated with small-to-medium-sized vessel vasculitis and differences from autoimmune myositis

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Abstract

Objective

Patients with systemic vasculitis may develop myalgia as an initial symptom. However, the immunopathology of vasculitic myopathy remains unclear. We investigated the immunopathological features of skeletal muscle in small-to-medium-sized vessel vasculitis.

Methods

We analysed muscle tissue biopsies from 15 patients with vasculitis, including antineutrophil cytoplasmic antibody-associated vasculitis and polyarteritis nodosa, and 15 patients with autoimmune myositis (AIM), including polymyositis and immune-mediated necrotising myopathy, as comparison disease controls. Immunohistochemical staining for CD56/neural cell adhesion molecule (NCAM), major histocompatibility complex class I, C5b-9/membrane attack complex (MAC), and CD31 was performed. The vascularity score was defined as the total number of CD31-expressing blood vessels. The association between CD56/NCAM-expressing myofibres and clinical findings was evaluated in patients with vasculitis.

Results

Patients with vasculitis had a significantly lower frequency of CD56/NCAM-expressing myofibres than those with AIM and a positive correlation between the frequency of CD56/NCAM-expressing myofibres and serum aldolase levels. Patients with vasculitis had significantly fewer major histocompatibility complex class I-expressing myofibres and C5b-9/MAC deposits on the sarcolemma than those with AIM. C5b-9/MAC deposits in blood vessels were observed in >70% of patients with vasculitis. Patients with vasculitis had significantly higher vascularity scores in the endomysium than those with AIM.

Conclusion

Patients with vasculitis demonstrated mild myofibre damage based on the lower involvement of CD56/NCAM-expressing myofibres compared to those with AIM. Complement component deposits on the vessel walls and hypervascularity in the endomysium areas may be immunopathological features of vasculitic myopathy.

Key words

vasculitis, myopathy, neural cell adhesion molecule, C5b-9, ANCA-associated vasculitis, polyarteritis nodosa

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Introduction

Histopathological assessment is an ideal procedure for the definite diagnosis of primary systemic vasculitis (PSV), including small-sized vessel vasculitis (SV) and medium-sized vessel vasculitis (MV). Moreover, determining the appropriate biopsy site that includes necrotising vasculitis for the histological diagnosis of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and polyarteritis nodosa (PAN) is important (1, 2). The consensus algorithm of the European Medicines Agency (EMA algorithm) (3), as well as newly validated criteria (4-7), have been useful in classifying microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), eosinophilic granulomatosis with polyangiitis (EGPA), and PAN using surrogate markers, even without histological findings of necrotising vasculitis. However, approximately 15% of patients presenting with ANCA positivity are classified as unclassifiable (8).

Myalgia is a common symptom and component of the disease activity index in patients with PSV (9, 10), although it is not included in the prevalent classification or diagnostic criteria for PSV (11). Muscular involvement could develop as the initial manifestation of PSV (12, 13). We recently reported that myalgia is frequently observed in the lower limbs, particularly in the early phases of MPA and PAN (13). Moreover, MPA and PAN are most frequently classified in patients with vasculitic myopathy (11). A musculoskeletal lesion is a potential biopsy site for detecting SV and MV when another suitable organ for biopsy cannot be found, and necrotising vasculitis was observed in 33-88% of patients who underwent muscle biopsy (13-16).

Autoimmune myositis (AIM) is a representative autoimmune disorder that targets muscle and is accompanied by musculoskeletal manifestations such as muscle weakness and an increase in serum creatine kinase (CK) levels (17). By contrast, patients with PSV develop myalgia without any accompanying musculoskeletal findings (13). However, objective evaluation of myopathy in patients with PSV, including its immunopathological features and myofibre damage, remains unknown.

In this study, we investigated the histopathological features of skeletal muscle in vasculitic myopathy by comparing the immunohistochemical (IHC) findings of muscle biopsy samples from patients with AIM.

Materials and methods

Patients and study design

This study included 15 patients with vasculitis and 15 with AIM as the disease control group who underwent muscle biopsies at our hospital between April 2014 and June 2022. All patients with vasculitis underwent a muscle biopsy for pathological diagnosis because myalgia was the principal symptom without another suitable biopsy site. The biopsy site was determined when magnetic resonance imaging findings of the targeted muscle indicated a hyperintense signal on short-tau inversion recovery (Fig. 1A). The classifications of MPA, GPA, EGPA, and PAN were determined based on the criteria of the Chapel Hill Consensus Conference (1), the EMA algorithm (3), and the 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria (4-6). Patients with AIM included 11 patients with polymyositis (PM), classified according to the European League Against Rheumatism and the American College of Rheumatology Classification Criteria (18), and four patients with immune-mediated necrotising myopathy (IMNM), which is a distinct subtype of AIM (19). IMNM is determined when haematoxylin-eosin (HE)-stained muscle sections indicated histological features including myofibre necrosis and regeneration (20). Of the four patients with IMNM, the test results for anti-signal recognition particle and anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase antibodies were positive in one and two patients, respectively, and one patient was negative for myopathy-specific antibodies. Clinical findings and IHC analyses of biopsied muscle specimens were compared between patients with vasculitis and those with AIM. Clinical findings just before muscle biopsy, including

white blood cell counts, serum CK and aldolase (ALD) levels, C-reactive protein, estimated glomerular filtration rate, and a manual muscle test in eight bilateral muscles (manual muscle testing-8 [MMT-8]) (21), were extracted from clinical records. In patients with vasculitis, the Birmingham Vasculitis Activity Score (version 3); the distribution of myalgia, defined as pain in the muscles (9, 22); and positivity for myeloperoxidase (MPO)- and proteinase 3 (PR3)-ANCA were also reviewed using their clinical records. This study was approved by the local ethics committee of Shinshu University (approval numbers: 3907 and 5675). All participants provided written informed consent.

IHC staining and quantitative evaluation

All patients with vasculitis underwent an open muscle biopsy of the quadriceps femoris or gastrocnemius, and all patients with AIM underwent an open muscle biopsy of the deltoid or quadriceps femoris. Biopsied muscle samples were treated as fresh frozen specimens in isopentane chilled with liquid nitrogen for staining. Unfixed 5-μm sections were stained with HE. Other 5-μm sections were fixed with pre-cooled acetone and blocked with 1% bovine serum albumin phosphate-buffered saline solution after eliminating endogenous peroxidase activity in 0.3% hydrogen peroxide phosphate-buffered saline for IHC staining. Blocked sections were incubated with the following antibodies for two hours: mouse anti-CD56/neural cell adhesion molecule (NCAM) (1:100; DAKO, 123C3), mouse anti-C5b-9/membrane attack complex (MAC) (1:50; DAKO, aE11), mouse anti-major histocompatibility complex-class I (MHC-I) (1:100; Neo-Markers, W6/32), or mouse anti-CD31 (1:500; DAKO, JC70A). Subsequently, the sections were incubated with goat anti-mouse IgG biotinylated antibody (1:500; DAKO, E0433) and developed using the VECTASTAIN ABC kit (Vector, PK-6100) and the ImmPACT 3,3'-diaminobenzidine substrate kit (Vector, SK-4105). The stained specimens were analysed using a BZ-X710 microscope system (KEYENCE). The frequencies of CD56/NCAM, C5b-9/MAC, and MHC-I-expressing myofibres were determined by calculating their ratios to the total number of stained and unstained myofibres detected in 10 different high-power fields (1.58 mm^2). C5b-9/MAC positivity was determined by its deposition on the sarcolemma and blood vessels without intracellular staining. The vascularity score was defined as the total number of blood vessels expressing CD31, an endothelial cell marker, and was used to evaluate angiogenesis (23-25) in 10 different high-power fields. The vascularity scores were evaluated by counting the CD31-expressing luminal structures in the endomysium and perimysium areas. Two investigators (SN and YS) performed blinded counts and calculations of IHC-stained samples and the vascularity scores.

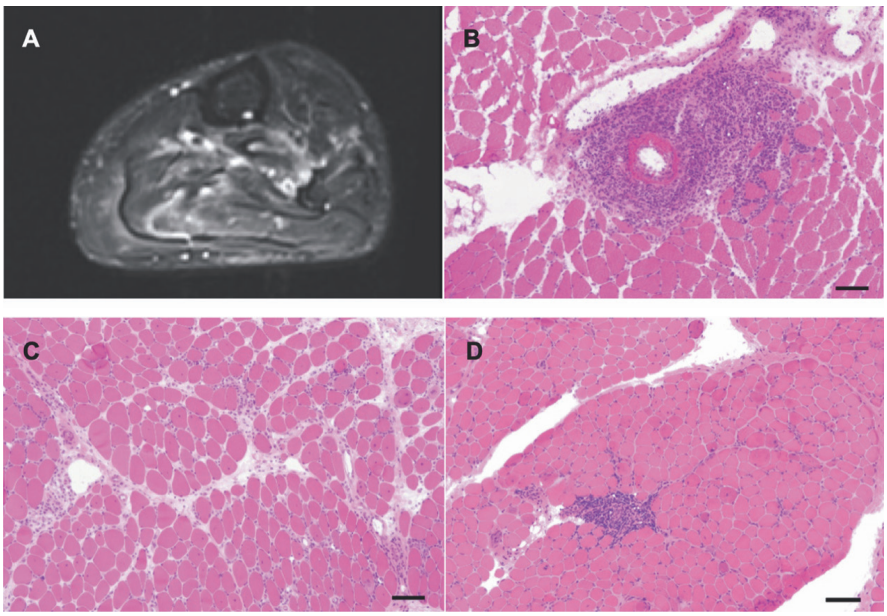

Fig. 1. Radiological and histopathological findings of involved skeletal muscles. Representative magnetic resonance imaging of the distal lower legs of a patient with vasculitis showing hyperintensity on short-tau inversion recovery (**A**). Representative histopathological findings of biopsied muscle stained with haematoxylin and eosin in a patient with vasculitis (**B**), polymyositis (**C**), and immune-mediated necrotizing myopathy (**D**). Scale bar: 100μm.

Statistical analysis

All data were presented as medians with interquartile ranges (IQR). *p*-values of <0.05 were defined as statistically significant. The Mann-Whitney U-test and Fisher's exact probability test were used to compare two independent groups. A Spearman's rank correlation coefficient test was performed to evaluate the relationship between the quantitative IHC data and clinical findings. Statistical analyses were performed using JMP software version 14.3.0 (SAS Institute Inc., Cary, NC, USA) and the BellCurve for Excel (SSRI, Tokyo, Japan).

Results

Pathological and clinical features of patients with vasculitis and comparisons with those with AIM

Twelve of the 15 patients with vasculitis whose biopsies were HE-stained had necrotising vasculitis (Fig. 1B), but three had non-necrotising vasculitis. Twelve patients presented with necrotising vasculitis included seven with MPA, three with GPA, and two with PAN. Of the three patients who presented with non-necrotising vasculitis, two were classified as having MPA, whereas one was classified as unclassifiable (Table I). MPO-ANCA positivity was observed in 12 patients with AAV, while the test results for ANCA were negative in three. All patients with vasculitis developed myalgia as an initial clinical episode of vasculitis. Myalgia in the distal lower legs was observed in all patients (100%) with vasculitis, and myalgia in the proximal lower legs was

Table I. Clinical characteristics of 15 patients with vasculitis.

Classification of vasculitis	
MPA, n (%)	9 (60)
GPA, n (%)	3 (20)
EGPA, n (%)	0 (0)
PAN, n (%)	2 (13)
Unclassifiable, n (%)	1 (7)
MPO-ANCA, n (%)	12 (80)
PR3-ANCA, n (%)	0
BVAS, median (IQR)	8.0 (5.5-15.0)
Myalgia, n (%)	15 (100)
Distribution of myalgia	
Neck, n (%)	3 (20)
Upper extremities, proximal, n (%)	7 (47)
Upper extremities, distal, n (%)	5 (33)
Lower extremities, proximal, n (%)	12 (80)
Lower extremities, distal, n (%)	15 (100)
Muscular symptomatic duration*, median months (IQR)	1.5 (1.0-3.0)
Fever, n (%)	9 (60)
Arthritis, n (%)	8 (53)
Weight loss, n (%)	4 (27)
Cutaneous, n (%)	4 (27)
Mucous membranes and eyes, n (%)	0
ENT, n (%)	4 (27)
Pulmonary, n (%)	10 (67)
Cardiovascular, n (%)	0
Abdominal, n (%)	0
Renal, n (%)	7 (47)
Nervous system, n (%)	2 (13)
Treatment before muscle biopsy	
Prednisolone, n (%); median mg/day (IQR)	3 (20); 5 (5-7)
Tacrolimus, n (%)	1 (7)
NSAID, n (%)	7 (47)

MPA: microscopic polyangiitis; GPA: granulomatosis with polyangiitis; EGPA: eosinophilic granulomatosis with polyangiitis; PAN: polyarteritis nodosa; BVAS: Birmingham Vasculitis Activity Score; IQR: interquartile range; ENT: ear, nose and throat; MPO: myeloperoxidase; PR3: proteinase 3; ANCA: anti-neutrophil cytoplasmic antibody; NSAID: non-steroidal anti-inflammatory drug.
*Period between the occurrence of myalgia and muscle biopsy.

observed in 12 patients (80%). Median period between the occurrence of myalgia and the performance of a muscle biopsy was 1.5 months (IQR, 1.0-3.0) in patients with vasculitis. Low doses of prednisolone were administered to three patients with vasculitis, in whom one patient concomitantly had tacrolimus, before muscle biopsy. MMT-8 was significantly higher in patients with vasculitis than in those with AIM ($p<0.0001$), although five patients with vasculitis had mild weakness in their proximal lower extremities (Table II).

Table II. Comparisons of clinical findings between patients with vasculitis and AIM.

	Vasculitis	AIM	*p*-value
Number	15	15	
Age, years, mean ± SD	70 ± 11	68 ± 7	0.119
Female:Male	10:5	12:3	0.682
MMT-8	150 (146-150)	135 (127-141)	< 0.0001
White blood cells, /μL	12290 (10325-14450)	5800 (5340-7570)	0.0002
*[1]Creatine kinase, U/L	20 (16-46)	1099 (8435-2655)	< 0.0001
*[2]Aldolase, U/L	4.4 (3.2-5.6)	17.9 (14.5-33.7)[†]	0.0001
C-reactive protein, mg/dL	10.02 (6.23-13.55)	0.11 (0.05-0.24)	< 0.0001
eGFR	74 (62-80)	106 (97-118)	0.0008

AIM: autoimmune myositis; MMT-8: manual muscle test-8 (21); eGFR: estimated glomerular filtration rate; SD: standard deviation.
Data are presented as the median (interquartile range [IQR]). A *p*-value of <0.05 was considered statistically significant.
*[1] normal value: <165 U/L; *[2] normal values: <6.1 U/L.
[†]The total number of patients who examined aldolase was 13 in AIM because of missing values.

Serum CK levels were within normal ranges in all patients with vasculitis, whereas ALD levels were higher than normal ranges in three patients (6.3, 8.9, and 7.9 U/L, respectively). Serum CK and ALD levels were significantly lower in patients with vasculitis than in those with AIM ($p<0.0001$ and $p=0.0001$, respectively). Significantly higher white blood cell counts, serum C-reactive protein levels, and lower estimated glomerular filtration rates were observed in patients with vasculitis than in those with AIM ($p=0.0002$, $p<0.0001$, and $p=0.0008$, respectively).

Evaluation of myofibre damage in vasculitic myopathy

CD56/NCAM-expressing myofibres were observed in the biopsied muscle sections from all patients with vasculitis and AIM (Fig. 2A-2D). The frequency of CD56/NCAM-expressing myofibres was significantly lower in patients with vasculitis than in those with AIM (median 1.2% [IQR, 0.88–9.42] *vs*. 19.8% [7.57–25.4]; $p=0.008$) (Fig. 2E). Of the total CD56/NCAM-expressing myofibres, the frequency of CD56/NCAM-expressing myofibres observed in the perifascicular areas was also evaluated as perifascicular-CD56/NCAM, resulting in no significant difference between patients with vasculitis and those with AIM (45.8% [IQR, 40.3-60.1] *vs*. 42.3% [37.9–47.1]; $p=0.281$) (Fig. 2F). The frequency of CD56/NCAM-expressing myofibres was significantly correlated with serum ALD levels in patients with vasculitis ($p=0.007$, Fig. 2G), although it did not correlate with other clinical findings, as shown in Table II (data not shown). The frequency of perifascicular-CD56/NCAM was significantly higher in patients with vasculitis who had decreased MMT-8 levels (n=5) than in those with normal MMT-8 levels (n=10) (64.7% [IQR, 52.2–75.0] *vs*. 43.4% [31.8-46.5]; $p=0.043$, Fig. 2H). In patients with AIM, neither the frequency of CD56/NCAM-expressing myofibres nor that of perifascicular-CD56/NCAM was significantly associated with clinical findings (data not shown). The frequency of CD56/NCAM-expressing myofibres was significantly lower in patients with AAV than in those with PM ($p=0.031$), whereas the frequency of perifascicular-CD56/NCAM was not significant different between two groups ($p=0.758$) (Supplementary Fig. S1).

Expression of MHC-I, C5b-9/MAC, and CD31 in vasculitic myopathy

MHC-I-expressing myofibres were observed in all patients with vasculitis and AIM, whereas MHC-I was more focally stained on the sarcolemma in patients with vasculitis than in those with AIM (Fig. 3A and 3B). The frequency of MHC-I-expressing myofibres was significantly lower in patients with vasculitis than in those with AIM (2.76% [IQR, 1.74–9.09] *vs*. 23.9% [9.45–55.4]; $p=0.003$) (Fig. 3C). C5b-9/MAC-expressing myofibres on the sarcolemma without intracellular staining were observed in five (33%) patients with vasculitis and in 12 (80%) with AIM ($p=0.025$). The frequency

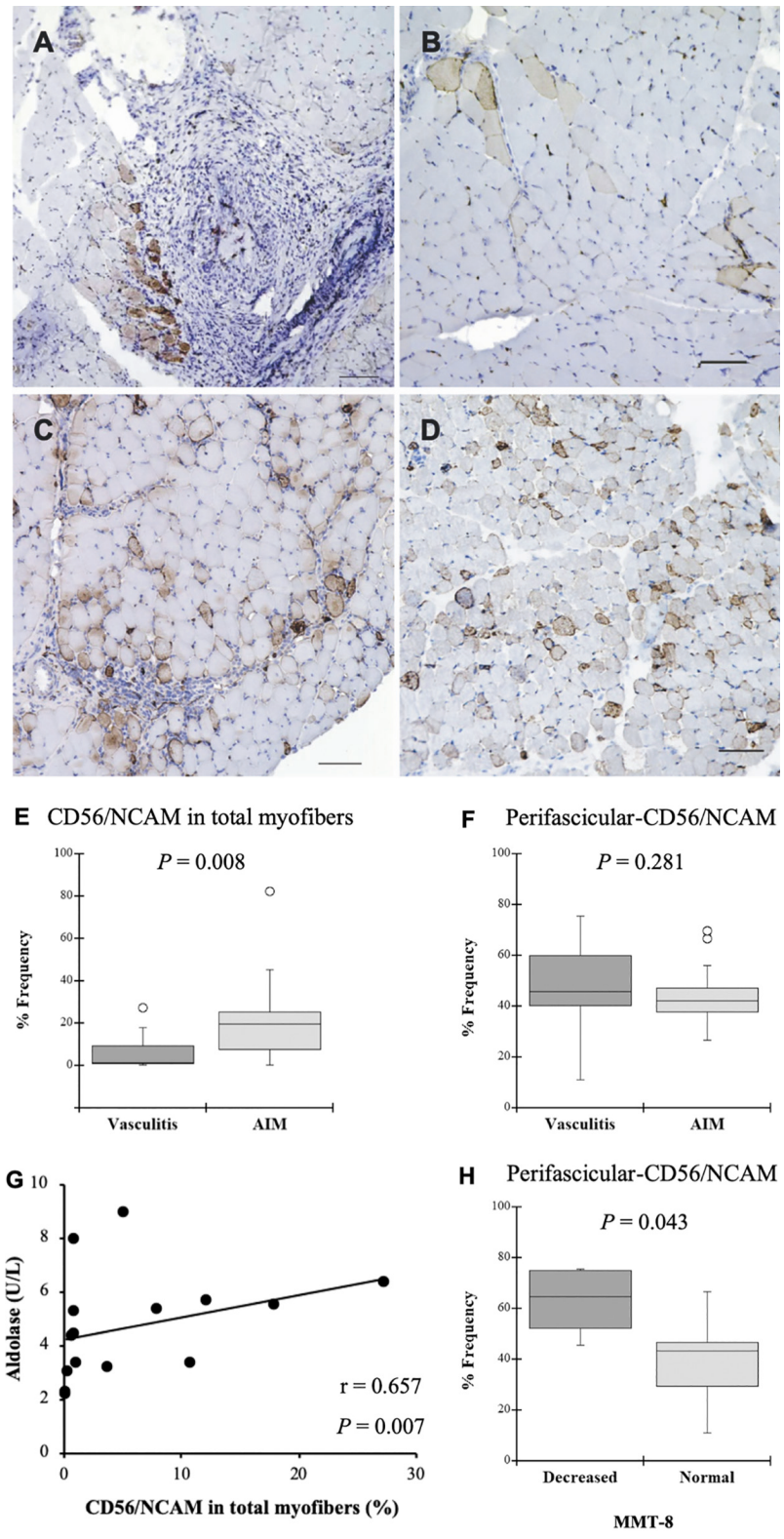

Fig. 2. CD56/NCAM-expressing myofibers. Representative immunohistochemical findings in patients with vasculitis (**A, B**) and AIM (**C, D**). The frequencies of CD56/NCAM-expressing myofibers in the total myofibres (**E**) and those of perifascicular-CD56/NCAM (CD56/NCAM-expressing myofibres of perifascicular areas in the total CD56/NCAM-expressing myofibers) (**F**) between patients with vasculitis and AIM. The linear correlation between frequencies of CD56/NCAM-expressing myofibres and serum aldolase levels in patients with vasculitis (**G**). Comparison of perifascicular-CD56/NCAM between patients with vasculitis having decreased MMT-8 (n=5) and normal MMT-8 (n=10) (**H**).
Scale bar: 100μm.
AIM: autoimmune myositis; MMT-8: manual muscle testing-8; NCAM: neural cell adhesion molecule.

of C5b-9/MAC-expressing myofibres was significantly lower in patients with vasculitis than in those with AIM (0% [IQR, 0–0.05] *vs*. 0.20% [0.04-0.39]; p=0.002) (Fig. 3D-3F), whereas the highest frequency of C5b-9/MAC-expressing myofibres was 0.72% in patients with AIM. C5b-9/MAC-expressing capillaries in the endomysium were observed in 11 (73%) patients with vasculitis and in 14 (93%) patients with AIM (p=0.165). C5b-9/MAC deposits on the blood vessel walls in the perimysium areas were observed in 12 (80%) of the patients with vasculitis and 13 (87%) of the patients with AIM (p=0.999). CD31-expressing vessels in both the endomysium and perimysium areas were observed in all patients with vasculitis and AIM. The vascularity scores in the endomysium areas were significantly higher in patients with vasculitis than in those with AIM (p=0.0004) (Fig. 3G-3I), whereas the vascularity scores in the perimysium areas were not significantly different between the two groups (p=1.000). The IHC findings for MHC-I, C5b-9, and CD31 expression were also compared between patients with AAV and those with PM (Supplementary Fig. S1). The frequency of MHC-I-expressing myofibres was significantly lower in patients with AAV than in those with PM (p=0.0002). C5b-9/MAC deposits on the sarcolemma were observed in four (33%) patients with AAV and in nine (82%) with PM (p=0.036). The frequency of C5b-9/MAC deposits on the sarcolemma was significantly lower in patients with AAV than in those with PM (p=0.009). The vascularity scores in the endomysium areas were significantly higher in patients with AAV than in those with PM (p=0.004).

Discussion

Patients with vasculitis, who had myalgia as the initial symptom of the disease, had less frequent muscle weakness and normal serum CK levels than those with AIM. Muscle weakness and elevated serum muscle enzyme levels are general clinical indicators of skeletal muscular damage that can be broadly elicited by traumatic events, mechanical stresses, and several muscular disorders

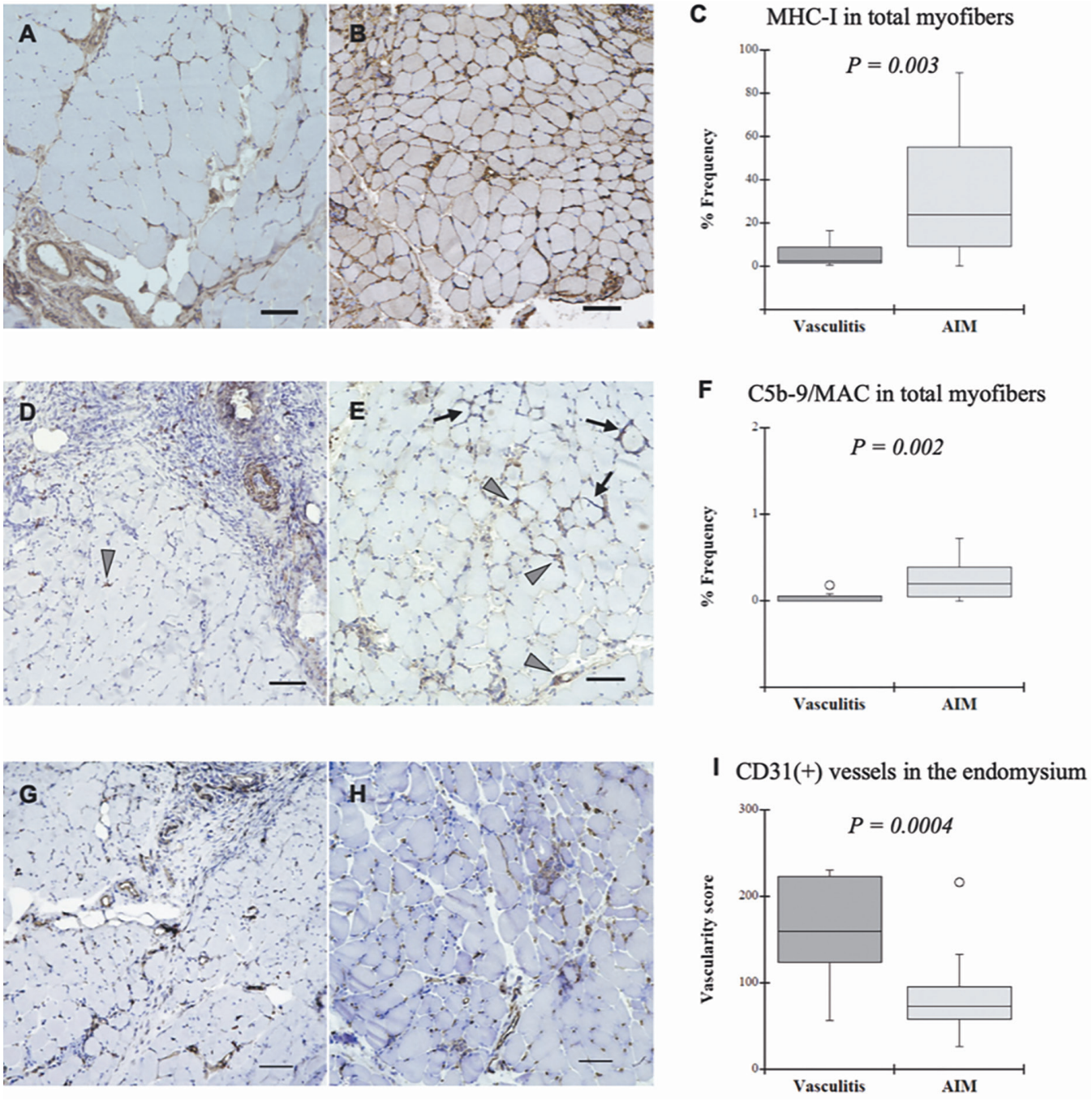

Fig. 3. MHC-I, C5b-9/MAC, and CD31 expression in biopsied muscles. Representative immunohistochemical findings of MHC-I, C5b-9/MAC, and CD31 in a patient with vasculitis (**A**, **D**, and **G**, respectively) and AIM (**B**, **E**, and **H**, respectively). Grey arrowheads display C5b-9/MAC-expressing blood vessels in the endomysium, and black arrows indicate C5b-9/MAC deposits on the sarcolemma. The frequencies of MHC-I-expressing myofibres (**C**) and C5b-9/MAC-expressing myofibres on the sarcolemma (**F**) in the total myofibers between patients with vasculitis and AIM. The vascularity scores in the endomysium areas between patients with vasculitis and those with AIM (**I**).
Scale bar: 100μm. AIM: autoimmune myositis; MAC: membrane attack complex; MHC-I: major histocompatibility complex class I.

(26). Notably, these muscular parameters are robustly associated with disease activity in patients with PM and IMNM (26-28), although they are not applicable for evaluating amyopathic dermatomyositis (DM) (26, 29). In quantitative analyses of CD56/NCAM-expressing myofibres, which is a marker for evaluating myofibre degeneration and regeneration (23), patients with vasculitis had a significantly lower frequency of CD56/NCAM-expressing myofibres than those with AIM, suggesting that mild myofibre damage is a pathological feature of vasculitic myopathy. Meanwhile, patients with vasculitis had a positive correlation between the frequency of CD56/NCAM-expressing myofibres and serum ALD levels, suggesting that a test for ALD may be useful for quantitatively evaluating myofibre damage in vasculitic myopathy when myalgia is merely involved in the absence of muscle weakness or elevated serum CK

levels. Elevated ALD levels may be associated with the pathological aspects of inflammation in perifascicular areas, which are predominantly observed in DM (30). Perifascicular atrophy is a specific and predominant morphology in DM and is pathologically distinct from other types of AIM (29, 31, 32). Small-to-medium-sized vessels, which supply capillaries and arterioles to myofibres as their upstream vascular systems in skeletal muscle tissues, are usually found in the perimysium area. This could explain why SV and MV cause inflammation and ischemic damage to myofibres adjacent to the perifascicular areas. Our results demonstrated a significantly higher frequency of perifascicular-CD56/NCAM in patients with vasculitis who had reduced MMT-8 levels than in those with normal MMT-8 levels, suggesting that myofibre damage around the perifascicular areas may predominantly affect myopathic symptoms in vasculitic myopathy. However, because the frequency of perifascicular-CD56/NCAM was not significantly different from that in patients with AIM, it was impossible to explain myofibre damage in the perifascicular region as a distinctive pathological feature of vasculitic myopathy. Additionally, it is necessary to estimate the cut-off serum ALD levels for evaluating myofibre damage in patients with vasculitis because serum ALD levels were within conventional normal values in the majority of patients. Taken together, our results suggest that mild myofibre damage is a pathological feature of vasculitic myopathy that is distinct from AIM. However, more research is needed to investigate the impact of myofibre damage on muscular weakness and serum ALD levels more precisely.

C5b-9/MAC deposits on the sarcolemma and endomysial capillaries are typical pathological features of IMNM and DM (17, 23, 31-34), whereas these pathological findings have also been observed in some patients who were classified as PM and anti-synthetase syndrome-associated myositis, respectively (23, 26, 34, 35). Our results demonstrated that patients with vasculitis had C5b-9/MAC deposits on the sarcolemma less frequently than those with AIM. However, C5b-9/MAC deposits on the endomysial capillaries were observed in 73% of patients with vasculitis, which was not significantly different from those with AIM. It has been suggested that C5b-9/MAC deposits on endomysial capillaries may indicate micro-vasculopathy (34). In addition, 80% of patients with vasculitis demonstrated C5b-9/MAC deposits on the MV and SV vessel walls in the perimysium areas. The pathology of AAV and PAN is fundamentally recognised as inflammatory and necrotising vasculitis in the absence of immune deposits (1, 36, 37). Conversely, complement activation has been implicated in the development of AAV (36), and significantly increased circulating C5b-9/MAC levels have been observed in patients with AAV (38, 39). Complement component deposition in involved organs, including the skin and kidneys, has also been observed in patients with AAV (40-42). Furthermore, C5b-9/MAC deposits were predominantly observed in cellular crescentic lesions in patients with ANCA-associated glomerulonephritis (42). Given the immunological and histological implications of C5b-9/MAC in the development of vasculitis, our results suggest that C5b-9/MAC deposits may be a pathological feature of targeted vessel walls in vasculitic myopathy. However, it needs to be clarified whether C5b-9/MAC deposits on blood vessels are merely a result of a focal immune response or can impact myofibre damage and muscular symptoms.

Increased angiogenesis in the muscular tissues of AIM has been demonstrated; notably, increased CD31-expressing capillaries in the endomysium were significantly observed in both PM and DM, whereas those in the perimysium were predominantly observed in DM (24, 25). Our results demonstrated significantly higher vascularity scores in patients with vasculitis than in those with AIM, suggesting that hypervascularity of capillaries in the endomysium areas is a pathological feature of vasculitic myopathy. Moreover, our results suggest that hypervascularity of the endomysium, despite mild myofibre damage, may lead to a reversible oedematous change of muscle tissues, resulting in abnormal signal intensity on muscle magnetic resonance imaging as a graphical feature of vasculitic myopathy (13, 43, 44). However, in this study, we only investigated CD31 expression to evaluate enhanced vascularity. Further research using other angiogenesis or vaso-permeability factors is required to elucidate the precise implications of vascularity in the development of vasculitic myopathy.

There are some limitations to the present study. This study did not include patients with EGPA and DM. Therefore, it may be insufficient to precisely elucidate the immunopathological features of vasculitic myopathy in AAV, and it is impossible to discriminate the immunopathological findings of vasculitic myopathy from those of DM. In addition, we employed myopathies related to not only AAV but also PAN and AIM as the comparison control which also heterogeneously included PM and IMNM. It may be ideal to use AAV, PAN, PM, or IMNM separately to identify different immunopathological features; however, the small numbers of PAN and IMNM were insufficient for statistical analysis in this study. Moreover, sarcolemmal deposits of not only C5b-9/MAC but also p62, specific myofibre stains for IMNM, should be statistically analysed by recruiting a larger number of IMNM samples in the next experimental plan for vasculitic myopathy. Predominant positivity for MPO-ANCA has been found in Japanese patients as a geographical feature of AAV; conversely, positivity for PR3-ANCA is predominantly observed in European countries (45). In fact, all three patients with GPA tested positive for MPO-ANCA in this study. Therefore, it is necessary to investigate the immunopathological findings in vasculitic myopathy by confounding ethnic and genetic factors.

In conclusion, a significantly lower frequency of CD56/NCAM-expressing myofibres than in patients with AIM, namely mild myofibre damage, was demonstrated in patients with vasculitis. C5b-9/MAC deposits were observed on the vessel walls in many patients with vasculitis, although they were hardly detected on the sarcolemma. Patients with vasculitis showed hypervascular-

ity in the endomysium areas, according to a higher frequency of CD31-expressing capillaries than those with AIM. Our results suggest that mild muscle damage, hypervascularity, and complement component deposits on blood vessels may be pathological features of vasculitic myopathy, whereas more precise mechanisms that cause changes in myofibres and blood vessels should be investigated in further studies.

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