Received: 22 September 2021 | Revised: 15 June 2022 | Accepted: 6 July 2022

DOI: 10.1111/nan.12840

ORIGINAL ARTICLE

Neuropathology and Applied Neurobiology
JOURNAL OF THE BRITISH NEUROPATHOLOGICAL SOCIETY

WILEY

# Capillary pathology with prominent basement membrane reduplication is the hallmark histopathological feature of scleromyositis

Benjamin Ellezam[1] | Valérie Leclair[2] | Yves Troyanov[3] | Imane Bersali[4] | Margherita Giannini[4] | Sabrina Hoa[5] | Josiane Bourré-Tessier[5] | Valérie Nadon[6] | Julie Drouin[7] | Jason Karamchandani[8] | Erin O'Ferrall[9] | Béatrice Lannes[10] | Minoru Satoh[11] | Marvin J. Fritzler[12] | Jean-Luc Senécal[5] | Marie Hudson[2] | Alain Meyer[4] | Océane Landon-Cardinal[5]

[1]Division of Pathology, CHU Sainte-Justine, Department of Pathology and Cell Biology, Université de Montréal, Montréal, Québec, Canada

[2]Division of Rheumatology, Jewish General Hospital, Department of Medicine, McGill University, Montréal, Québec, Canada

[3]Division of Rheumatology, Hôpital du Sacré-Coeur, Department of Medicine, Université de Montréal, Montréal, Québec, Canada

[4]Service de physiologie - explorations fonctionnelles musculaires, service de rhumatologie et Centre de référence des maladies autoimmunes rares, Hôpitaux universitaires de Strasbourg, Strasbourg, France

[5]Division of Rheumatology, Centre hospitalier de l'Université de Montréal (CHUM), Autoimmunity Research Laboratory, CHUM Research Center; Department of Medicine, Université de Montréal, Montréal, Québec, Canada

[6]Division of Rheumatology, Hôpital Notre-Dame, Department of Medicine, Université de Montréal, Montréal, Québec, Canada

[7]Division of Rheumatology, Centre Hospitalier Affilié Universitaire Régional (CHAUR) du CIUSSS Mauricie Centre-du-Québec, Department of Medicine, Université de Montréal, Montréal, Québec, Canada

[8]Department of Pathology, Montreal Neurological Institute and Hospital, Montréal, Québec, Canada

[9]Department of Neurology and Neurosurgery and Department of Pathology, McGill University and the Montreal Neurological Institute and Hospital, Montréal, Québec, Canada

[10]Service de Pathologie, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

[11]Department of Clinical Nursing, University of Occupational and Environmental Health, Kitakyushu, Japan

[12]Department of Medicine, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

**Correspondence**
Océane Landon-Cardinal, Division of Rheumatology, Centre hospitalier de l'Université de Montréal, 1051 rue Sanguinet, Montréal, Québec H2X 3E4, Canada.
Email: oceane.landon-cardinal@umontreal.ca

Benjamin Ellezam, Division of Pathology, CHU Sainte-Justine, 3175 chemin de la Côte-Sainte-Catherine, Montréal, Québec H3T 1C5, Canada.
Email: benjamin.ellezam@umontreal.ca

**Funding information**
This study was supported by a Myositis Canada grant (OLC) and by Sclérodermie Québec (OLC, SH, JLS). JLS holds the University of Montreal Scleroderma Research Chair.

## Abstract

**Aims:** We aim to perform ultrastructural and histopathological analysis of muscle biopsies from a large group of systemic sclerosis (SSc) patients, including some with early/mild SSc features, and examine whether capillary pathology differentiates 'scleromyositis' (SM) from other auto-immune myositis (AIM) subsets.

**Methods:** Muscle biopsies from a total of 60 SM patients and 43 AIM controls from two independent cohorts were examined by electron microscopy, collagen-4 immunofluorescence (Col4IF) and routine light microscopy.

**Results:** Ultrastructural examination revealed prominent capillary basement membrane (BM) reduplication (4+ layers in >50% of capillaries) in 65% of SM vs 0% of AIM controls ($p < 0.001$). In SM cases without prominent BM reduplication, capillary dilation was the most distinctive feature, present in 8% of capillaries in SM vs 2% in controls ($p = 0.001$). Accumulation of ensheathed pericyte processes was another characteristic feature of SM and closely correlated with the degree of BM reduplication ($r = 0.833$, $p < 0.001$).

  

On light microscopy, BM marker Col4IF revealed more frequent capillary enlargement in SM than in controls (84% vs 21%, $p < 0.001$). SM cases were classified as non-inflammatory myopathy (36%), non-specific myositis (33%) or immune-mediated necrotizing myopathy (31%), but despite this histopathological heterogeneity, prominent BM reduplication remained a constant finding. In the 16 SM patients with early/mild SSc features, 63% showed prominent BM reduplication.

**Conclusions:** These results show that capillary pathology, and in particular prominent capillary BM reduplication, is the hallmark histopathological feature of SM even in patients with early/mild SSc and support the concept of SM as an organ manifestation of SSc and a distinct subset of AIM.

**KEYWORDS**
auto-immune myositis, capillaries, electron microscopy, muscle biopsy, pericytes, scleroderma, systemic sclerosis, vasculopathy

## INTRODUCTION

Myositis has been reported in 5%–96% of systemic sclerosis (SSc, scleroderma) patients [1], a broad range of prevalence that highlights the absence of a standard clinical and histopathological definition of myositis in SSc. Myositis was in fact not selected as an SSc feature in the elaboration of the 2013 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) SSc classification criteria, and instead was considered an SSc mimicker [2]. Meeting the ACR/EULAR classification criteria for both SSc [3] and auto-immune myositis (AIM) [4] has subsequently been proposed as a definition of SSc-myositis overlap syndrome [5], but it is limited by low sensitivity [6]. Indeed, SSc patients with myositis may lack the pathognomonic clinical criterion of SSc (i.e. skin thickening) or may have myositis-associated SSc autoantibodies other than those included in the SSc criteria (anti-centromere, anti-topoisomerase 1, anti-RNA polymerase III) [7]. We have thus proposed defining muscle involvement in SSc as scleromyositis (SM) to capture the full spectrum of this condition including patients who present predominantly with skeletal muscle disease and only mild SSc features [7].

An integrated approach that correlates histopathological findings with clinical and serological features has recently helped identify novel and more homogeneous subsets of AIM [8]. SM, however, is currently not recognised as a distinct histopathological subset by the European NeuroMuscular Center (ENMC) [9]. Recent studies have described SSc-associated muscle pathology as a heterogenous group, either conforming to various existing ENMC categories [10] or to proposed patterns including inflammatory SSc-associated myopathy [11], fibrosing myopathy [12,13] or minimal myositis with capillary pathology [14], but have not suggested unifying features to support SM as a distinct subset despite its histopathological heterogeneity.

Vasculopathy is a cardinal pathophysiological process of SSc [15], epitomised clinically by early-onset Raynaud's phenomenon, and has been reported on muscle biopsy in the form of capillary dropout, abnormal expression of pro- and anti-angiogenic factors, and thickening and lamination of basement membrane (BM) with endothelial swelling in a series of 35 patients with SSc-associated myopathy [16]. Legacy ultrastructural studies dating back to the 1960s had already described a characteristic pattern of capillary basement membrane (BM) reduplication in SSc-associated myopathy [17–19], but this finding remained overlooked. Recently, a set of characteristic ultrastructural capillary changes including BM reduplication was reported in a cohort of 18 patients with SSc and minimal muscle inflammation on light microscopy [14]. We have made similar observations in our own patients many with only mild clinical and less common serological

### Key Points

- A total of 60 scleromyositis and 43 auto-immune myositis controls were analysed by electron microscopy and revealed prominent capillary basement membrane reduplication in 65% of scleromyositis and 0% of controls.
- Prominent basement membrane reduplication was a constant feature of scleromyositis across all its heterogeneous histopathological patterns, supporting the concept of scleromyositis as a distinct myositis subset.
- This hallmark feature of scleromyositis was also frequent in patients with early/mild systemic sclerosis, most often presenting with Raynaud's phenomenon but without skin involvement, suggesting that myositis is an organ manifestation of systemic sclerosis rather than an associated disease.
- Electron microscopy is an essential tool to support an early diagnosis of scleromyositis.

SSc features. This has led us further to propose the concept of myositis as a distinct end-organ manifestation of SSc rather than an associated AIM [20].

To improve the identification of SM patients presenting with a wider range of SSc clinico-serological features than those included in the 2013 ACR/EULAR SSc classification criteria, we explored, using a larger number of patients and a broad range of AIM controls, whether capillary changes including BM reduplication on muscle biopsy could facilitate accurate diagnosis and validate SM as a distinct subset of AIM.

## MATERIALS AND METHODS

### Patient cohorts and clinical data

Muscle biopsies of the discovery cohort were from patients in the Canadian Inflammatory Myopathy Study (CIMS) registry; patients of the validation cohort were from the Strasbourg (France) myositis cohort. Patients were classified as SSc using the 2013 ACR/EULAR criteria [3]. For patients not fulfilling the ACR/EULAR criteria (i.e. mild SSc features), a diagnosis of SSc was made by a consensus of ≥2 experts based on clinico-serological features (e.g. Raynaud's phenomenon, SSc-type capillaroscopy, or SSc autoantibodies other than those included in the SSc criteria) [7]. All these patients had muscle involvement including muscle weakness, creatine kinase (CK) elevation (>200 IU/L) and/or myopathic electromyogram (EMG) and were thus identified as having SM. Disease duration at the time of muscle biopsy was calculated from onset of first manifestation attributable to the disease (either non-Raynaud SSc manifestation or myositis). Seronegative SM was defined as SM with no known SSc-specific (anti-centromere, -topoisomerase 1, -RNA polymerase III, -Th/To, -fibrillarin), or SSc-associated (anti-PM/Scl, -U1RNP, -Ku) autoantibodies. No anti-MDA5 or anti-synthetase antibodies were identified in seronegative SM. Scleroderma sine scleroderma was defined according to Poormoghim's criteria [21]. Controls included dermatomyositis (DM) and immune-mediated necrotizing myopathy (IMNM) according to ENMC clinico-sero-pathological criteria [9,22,23], inclusion body myositis (IBM) according to Lloyd's criteria [24], and anti-synthetase syndrome (ASS) defined using an integrated clinico-sero-pathological approach [8]. All muscle biopsies from the discovery cohort and control biopsies from the validation cohort were performed at hospitals in Quebec, Canada and processed at two neuromuscular pathology referral centres in Montreal (CHU Sainte-Justine and Montreal Neurological Institute). Biopsies of SM cases in the validation cohort were performed and initially processed in Strasbourg, France, and transferred to Montreal for further processing.

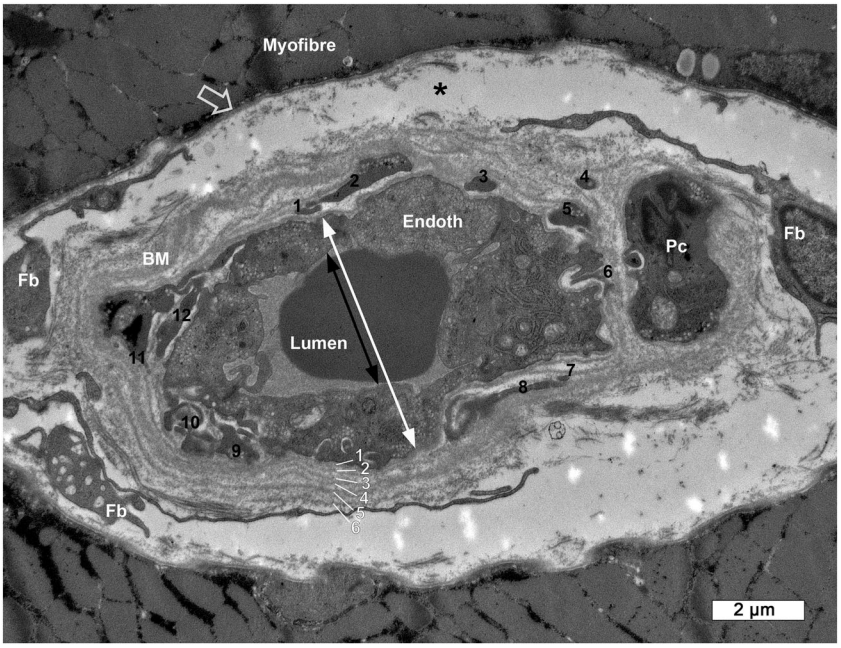


**FIGURE 1** Representative scleromyositis capillary showing ultrastructural anatomy and morphometric methodology. External endothelial diameter (white arrow) and luminal diameter (black arrow) were measured on the shortest line bisecting the capillary cross-section to control for potential effect of tangential cutting. Concentric basement membrane (BM) layers were counted (white numbers) at different areas around the capillary and when the number varied, the most representative was noted. Pericyte cell body (pc) is shown on the right. All distinct pericyte processes ensheathed within BM layers were counted (black numbers). Fibroblasts (fb) with long pseudopods and no pericellular BM deposition are shown around the capillary in the artefactually expanded endomysial space (black asterisk). Note the myofibre and its thin BM (open arrow) lining the sarcolemmal membrane. On light microscopic staining of capillaries, collagen type 4 immunofluorescence (Col4 IF) only stains BM and CD31 only stains endothelial cells (Endoth). Because capillary BM is enriched in Col4 compared with myofibre BM, capillaries on Col4 IF appear brighter than sarcolemmal membranes. Quadriceps, SM patient #5. Bar, 2 µm

### Ultrastructural evaluation

Ultrastructural evaluation was performed on 100 mesh nickel grids using a Philips EM208S transmission electron microscope and a 4-megapixel camera (AMT, Woburn, MA). For each case, the resin block with transversally oriented muscle and the highest number of capillaries as examined on thick sections was the one selected for grid preparation. Consecutive ultramicrographs were obtained of all capillaries encountered on the grid up to a maximum number of 50 in the discovery cohort and 30 in the validation cohort, considering that in cases with capillary dropout or with suboptimal archival tissue quality, the total count of capillaries present on the grid was lower than those intended maximum numbers. Ultramicrographs were examined in a consensus approach by a neuromuscular pathologist (BE) and a rheumatologist (OLC) with experience in neuromuscular pathology and the following parameters were noted for each capillary: luminal diameter, external diameter (without BM), number of BM layers, number of pericyte processes ensheathed by BM layers, evidence of endothelial activation (e.g. pale granular or swollen cytoplasm, lysosomal or other osmiophilic density accumulation, luminal collapse or capillary wall extreme thinning or gaps), and presence of diffusely thickened BM (>300 nm) without fine layering. Based on our preliminary study [20] and on the data of Siegert et al [14], 4+ layers ('marked') BM reduplication was selected as the best cut-off value to compare capillaries in SM cases and AIM controls. Cases with 'prominent' BM reduplication were defined as having 4+ layer BM reduplication in most (>50%) capillaries. Dilated capillaries were defined as having a luminal-to-external diameter ratio ≥90% and collapsed capillaries as having a ratio ≤15%. Methodology of these ultrastructural measurements and observations is further described in Figure 1.

### Light microscopy analysis

On cases with frozen material of sufficient quality, all stains recommended by the ENMC for histological assessment of inflammatory muscle biopsy [25] were performed. Antibodies used for immunohistochemistry included major histocompatibility complex class 1 (MHC1) (clone W6/32, 1:100 dilution), major histocompatibility complex class 2 (MHC2) (TAL-IB5, 1:200), C5b-9 (aE11, 1:100), Myxovirus Resistance A (MxA) (C-1, 1:10) (validation cohort only), CD31 (JC70A, Dako prediluted), CD4 (4B12, Dako prediluted), CD8 (C8/144B, Dako prediluted), CD20 (L26, Dako prediluted) and collagen type 4 (Col4) (clone CIV22, 1:1500) using a Link 48 Dako autostainer (Agilent, Santa Clara, CA). Immunofluorescence (IF) for Col4 (1:20) was performed manually.

Muscle biopsies were classified according to adapted ENMC histopathologic criteria [9,12] using a consensus approach by BE and OLC. Histopathologic features were scored using a grading system (Table S4A). Evaluation of capillary dropout and enlargement by Col4IF was performed using a consensus approach by BE and OLC, blinded to the clinical diagnosis. Dropout was considered significant when the number of myofibres in a fascicle exceeded the number of capillaries, and enlargement when most capillaries in a fascicle had an internal diameter double that of normal controls.

### Statistical analysis

Descriptive statistics were used to summarise the data. Variables were reported as numbers and/or percentages and quantitative variables were reported as means ± standard deviation or (range). Fischer's Exact Test was used to compare two categorical variables. Shapiro–Wilk test was used to assess distribution normality of continuous variables. Mann–Whitney U or Kruskal-Wallis tests were used to compare variables without normal distribution in 2 or 3+ groups, respectively, and Student t-test or one-way ANOVA, when the distribution was normal. ROC analysis was used to determine the discriminative power of ultrastructural biomarkers of SM and to determine optimal cut-off values. Data were analysed using IBM SPSS version 28.0. All tests were conducted at the 0.05 significance level and two-sided *p*-values were reported.

## RESULTS

### Patient characteristics

The discovery and validation cohorts included 32 and 28 SM cases, respectively. Patient characteristics for each cohort are shown in Table 1. In the pooled cohorts, patients were predominantly females (72%) and the mean age was 54 years (range 22–81). Mean disease duration at the time of muscle biopsy was 3.2 years (range 0–30). Muscle biopsy was performed because of proximal muscle weakness (77% of cases), CK elevation (95%; mean, 2130 IU/L; range, 63–10,209), and/or myopathic EMG (88%). Seventy-three per cent of patients fulfilled the 2013 ACR/EULAR SSc criteria [3]. In the remaining 16 patients (27%), the available features supporting the diagnosis of SSc included: positive antinuclear antibodies in 13 of 13 patients (100%), Raynaud's phenomenon in 12 of 16 (75%), interstitial lung disease in 8 of 16 (50%), SSc-associated autoantibodies in 8 of 15 (53%), SSc nailfold capillaroscopy in 10 of 14 (71%), puffy fingers in 6 of 16 (38%) and lower oesophageal dysmotility in 4 of 16 (25%) (Table S1). At the time of biopsy, disease duration was significantly shorter in patients not fulfilling the ACR/EULAR SSc criteria (0.90 vs 4.0 years, $p = 0.015$). Forty per cent of patients presented sine scleroderma. Controls included 43 cases (10 DM, 13 IBM, 11 IMNM and 9 ASS).

### Ultrastructural examination reveals abnormal capillary morphology in SM

On electron microscopy (EM), SM cases often showed enlarged capillaries with several fine layers of BM, large numbers of ensheathed pericyte processes and endothelial cell changes consistent with

**TABLE 1** Scleromyositis patient characteristics

| | Discovery cohort ($n = 32$) | Validation cohort ($n = 28$) |
|---|---|---|
| **Demographics** | | |
| Mean age at myositis diagnosis, years (range) | 54 (23–76) | 54 (22–81) |
| Female sex, *n* (%) | 24 (75) | 19 (68) |
| **Myopathic features** | | |
| Proximal muscle weakness, *n* (%) | 28 (88) | 18 (64) |
| Neck flexors weakness, *n* (%) | 21 (66) | 2/25 (8) |
| Head drop, *n* (%) | 8 (25) | 4/25 (16) |
| Distal weakness, *n* (%) | 15 (47) | 5/26 (19) |
| Dysphagia, *n* (%) | 12 (38) | 8 (29) |
| Myocarditis, *n* (%) | 2 (6) | 5 (18) |
| Mean peak serum CK level, IU/L (range) | 1944 (63–10,209) | 2284 (180–9239) |
| Myopathic EMG, *n* (%) | 26/28 (93) | 18/22 (82) |
| **SSc features** | | |
| ACR/EULAR SSc features | | |
| Raynaud's phenomenon, *n* (%) | 28 (88) | 23 (82) |
| Puffy fingers, *n* (%) | 8 (25) | 8 (29) |
| Sclerodactyly, *n* (%) | 19 (59) | 17 (61) |
| Digital ulcers, *n* (%) | 6 (19) | 6 (21) |
| Fingertip pitting scars, *n* (%) | 4/31 (13) | 5 (18) |
| Telangiectasias, *n* (%) | 11 (34) | 13 (46) |
| Abnormal nailfold capillaroscopy, *n* (%) | 23/26 (88) | 20/26 (77) |
| Interstitial lung disease, *n* (%) | 15 (47) | 15 (54) |
| Pulmonary arterial hypertension, *n* (%) | 1/31 (3) | 1 (4) |
| **SSc skin involvement** | | |
| Limited, *n* (%) | 7 (22) | 13 (46) |
| Diffuse, *n* (%) | 12 (37) | 4 (14) |
| Sine scleroderma, *n* (%) | 13 (41) | 11 (39) |
| **ACR/EULAR SSc criteria** | | |
| Definite SSc, *n* (%) | 23 (72) | 21 (75) |
| Non-definite SSc, *n* (%) | 9 (28) | 7 (25) |
| -Sine scleroderma, *n* (% of non-definite SSc) | 9/9 (100) | 7/7 (100) |
| **Serological features** | | |
| Positive ANA, *n* (%) | 29 (91) | 26/26 (100) |
| SSc-specific autoantibody, *n* (%) | 4 (12) | 3/27 (11) |
| SSc-associated autoantibody, *n* (%) | 12 (38) | 16/27 (59) |
| Seronegative (no known autoantibody specificities), *n* (%) | 16 (50) | 8/27 (30) |

Abbreviations: aAbs, autoantibodies; ACR/EULAR, American College of Rheumatology/European League Against Rheumatism; ANA, antinuclear antibody; CK, creatine kinase; EMG, electromyogram; SSc systemic sclerosis.

activation or injury (Figure 2A). In some cases, endothelial injury was severe and capillaries consisted almost exclusively of residual pericyte processes or debris among a trail of concentric layers of BM (Figure 2B). In other cases, capillaries did not show BM reduplication or increased numbers of pericyte processes but were thin walled with severely dilated lumina (Figure 2C). Some controls, mostly IBM, showed BM reduplication, increased numbers of pericyte processes and endothelial activation (Figure 2D), whereas others, often IMNM, instead showed diffuse thickening of BM without fine layering and with few ensheathed pericyte processes (Figure 2E).

## Detailed ultrastructural capillary analysis differentiates SM cases from other AIMs

To characterise the range, frequency and specificity of ultrastructural capillary pathology in SM, we performed detailed observations and

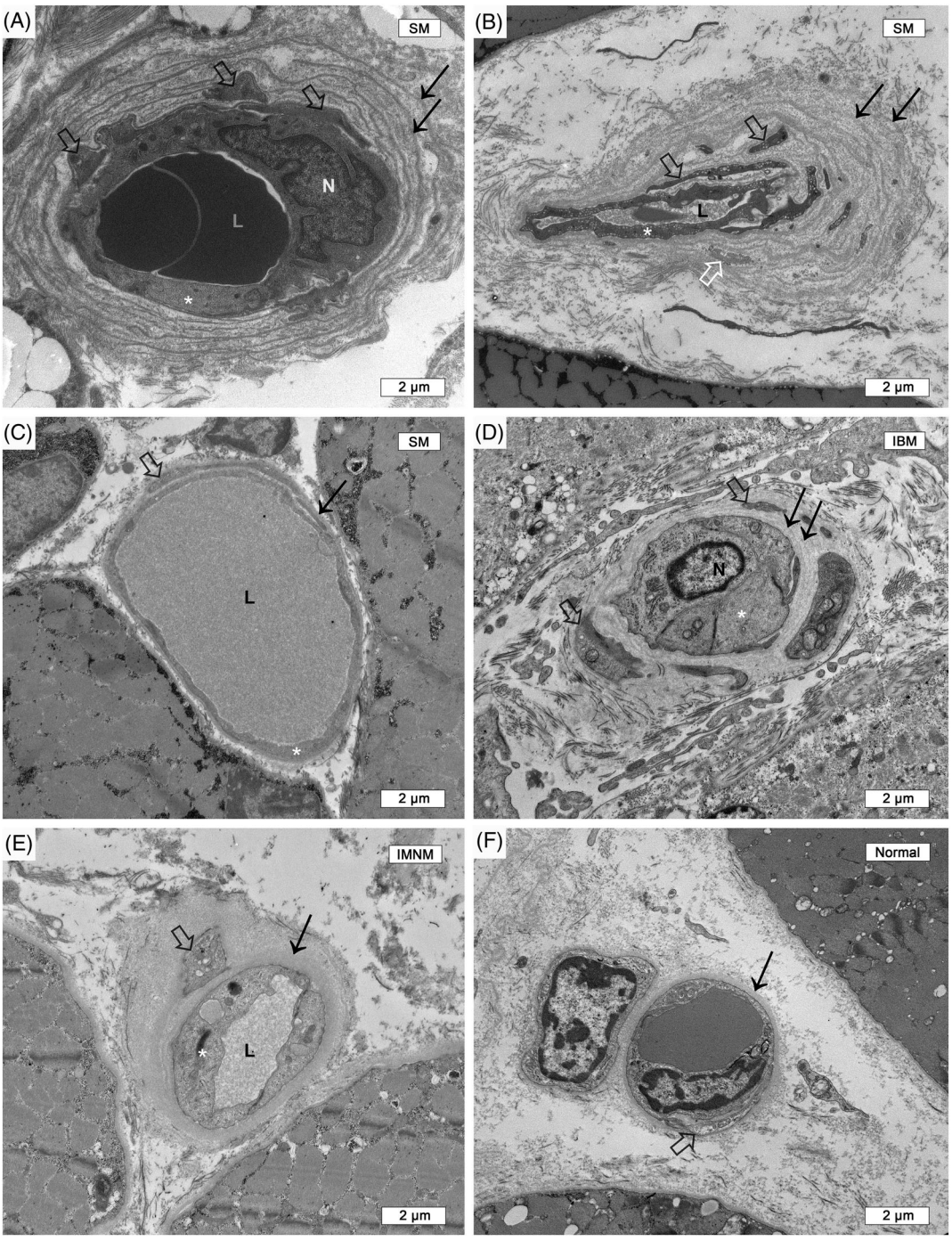


**FIGURE 2** Electron microscopy of endomysial capillaries in SM and controls. (A) Enlarged capillary with fine layered BM reduplication (black arrows, ~7 layers), increased number of ensheathed pericyte processes (open arrows) and a pale granular area of endothelial cell cytoplasm (asterisk) suggesting activation (patient #6, quadriceps, L, lumen, N, nucleus). (B) Collapsed end-stage capillary with fine layered BM reduplication (black arrows, ~8 layers), extreme thinning of endothelial cell (asterisk), and frequent ensheathed pericyte processes (black open arrows) or debris (white open arrow) (patient #7, quadriceps, L, lumen). (C) Dilated capillary with extreme thinning of endothelial cell (asterisk) and without BM reduplication (black arrow) or increased number of pericyte processes (open arrow) (patient #59, deltoid, L, lumen). (D) Collapsed capillary with thin-layered BM reduplication (black arrows, ~5 layers), frequent ensheathed pericyte processes (open arrows), endothelial cell oedema (asterisk) and absence of visible lumen (IBM control #2). (E) Capillary of normal diameter with endothelial oedema and osmiophilic densities (asterisk), diffusely thickened BM without thin layering (black arrow) and no increase in number of ensheathed pericyte processes (open arrow) (IMNM control #24 with type 2 diabetes). (F) Normal capillary with thin single layer of BM (black arrow) and a single ensheathed pericyte process (open arow) (quadriceps, 43-year-old male with mild neurogenic atrophy). Bars: 2 µm. BM, basement membrane; IBM, inclusion body myositis; IMNM, immune-mediated necrotizing myopathy; SM, scleromyositis

measurements of capillaries (range 20–50 per case, average 35) in the 32 SM cases and 18 AIM controls of our discovery cohort (Table 2 and Table S2A-B). Capillaries with 4+ layers BM reduplication were encountered in both SM cases and AIM controls but the mean proportion of affected capillaries on each grid was much higher in SM (47% vs 6%, $p < 0.001$) (Table 2). When only considering cases where >50% of capillaries on the grids showed 4+ layers BM reduplication, this feature was present in 59% of SM cases and none of AIM controls ($p < 0.001$) (Table 3 and Table S2A-B). Both the mean and maximum number of BM layers per capillary were also higher in SM compared with controls (3.7 vs 1.9, $p < 0.001$ and 6.7 vs 3.7, $p < 0.001$, respectively). Some capillaries showed diffuse thickening of BM (>300 nm) without fine layering, but these were significantly less frequent in SM than in controls (2% vs 17%, $p = 0.048$). This cohort included only one patient with known type 2 diabetes (an IMNM control), and that biopsy showed diffuse thickening of BM in 100% of capillaries. Another ultrastructural observation that was more frequent in SM cases than in controls was the mean number of ensheathed pericyte processes per capillary (5.1 vs 2.8, $p < 0.001$) but that parameter did not appear to be independent from the degree of BM reduplication as it strongly correlated with the mean number of BM layers per capillary ($r = 0.833$, $p < 0.001$) (Figure S1). Other parameters that were numerically more frequent in SM cases compared with controls included proportions of dilated or collapsed capillaries or proportion of capillaries with endothelial activation, although this did not reach statistical significance (Table 2). A ROC analysis performed to compare the ability of the different ultrastructural parameters to predict SM revealed similar area under the curve (AUC) values but the proportion of capillaries with 4+ layers BM reduplication was the parameter with the most left-skewed curve (maximising specificity) and with the most practical cut-off value (50%) for evaluation in routine clinical practice by a pathologist (Figure 3A). At that cut-off value, where >50% capillaries showed 4+ layers BM reduplication (from then on referred to as 'prominent BM reduplication'), this parameter had a sensitivity of 59% and a specificity of 100% to differentiate SM cases from AIM controls (Table S3).

To confirm these findings, we performed the same morphometric analysis on 28 SM cases and 25 AIM controls from an independent validation cohort (Table S2A-B). Again, now based on an average of 22 capillaries per case (range 15–30), the same ultrastructural parameters were significantly higher in SM compared with controls (Table 2) and ROC analysis provided similar curves and cut-off values (Figure S2). Prominent BM reduplication had a sensitivity of 71% and a specificity of 100% to differentiate SM cases from AIM controls (Table S3).

To increase significance in all further clinical and pathologic subgroup analyses, cases ($n = 60$) and controls ($n = 43$) from the two cohorts were pooled (Table 2). Prominent BM reduplication had an overall sensitivity of 65% and specificity of 100% to identify SM cases (Table S3). When using the number of ensheathed pericyte processes as an alternative parameter to detect SM cases, the optimal cut-off value of ≥4 offered a sensitivity of 67% and specificity of 91%. In the pooled cohorts, there was now a statistically significant higher

**TABLE 2** Ultrastructural parameters of capillaries in scleromyositis cases and auto-immune myositis controls

| Features | Discovery cohort | | | Validation cohort | | | Pooled cohorts | | |
|---|---|---|---|---|---|---|---|---|---|
| | Scleromyositis $n = 32$ | Controls $n = 18$ | *p*-value | Scleromyositis $n = 28$ | Controls $n = 25$ | *p*-value | Scleromyositis $n = 60$ | Controls $n = 43$ | *p*-value |
| % of capillaries with 4+ layers BM reduplication | 47 (0–100) | 6 (0–33) | **<0.001** | 58 (0–100) | 9 (0–28) | **<0.001** | 52 (0–100) | 8 (0–33) | **<0.001** |
| Mean number of BM layers per capillary | 3.7 (1.2–7.6) | 1.9 (1.1–4.0) | **<0.001** | 3.8 (1.3–8.6) | 2.1 (1.0–2.9) | **<0.001** | 3.7 (1.2–8.6) | 2.0 (1.0–4.0) | **<0.001** |
| Maximum number of BM layers per capillary | 6.7 (2–14) | 3.7 (2–6) | **<0.001** | 5.9 (2–15) | 3.2 (1–7) | **<0.001** | 6.3 (2–15) | 3.7 (1–7) | **<0.001** |
| % of capillaries with thick BM (>300 nm) without fine layering | 2 (0–15) | 17 (0–100) | **0.048** | 10 (0–87) | 36 (0–100) | **0.005** | 6 (0–87) | 28 (0–100) | **<0.001** |
| Mean number of ensheathed pericyte processes per capillary | 5.1 (2.2–11.0) | 2.8 (0.8–6.0) | **<0.001** | 4.9 (2.5–9.0) | 2.6 (0.7–4.4) | **<0.001** | 5.3 (2.2–11) | 2.7 (0.7–6.0) | **<0.001** |
| % of capillaries with endothelial activation | 80 (45–100) | 67 (17–100) | n.s. | 61 (17–100) | 68 (19–100) | n.s. | 76 (17–100) | 68 (17–100) | n.s. |
| Luminal diameter (μm) | 2.5 ± 0.8 | 2.9 ± 0.8 | n.s. | 2.0 ± 0.5 | 2.3 ± 0.7 | n.s. | 2.5 ± 0.8 | 2.4 ± 0.7 | n.s. |
| External diameter (μm) | 4.3 ± 0.8 | 3.6 ± 0.8 | **0.014** | 3.7 ± 0.8 | 4.1 ± 0.8 | n.s. | 4.0 ± 0.9 | 3.9 ± 0.9 | n.s. |
| % of dilated capillaries | 7.1 (0–39) | 2.4 (0–15) | n.s. | 2.6 (0–14) | 2.2 (0–33) | n.s. | 5.2 (0–39) | 2.3 (0–33) | **0.011** |
| % of collapsed capillaries | 4.6 (0–25) | 2.9 (0–15) | n.s. | 8.8 (0–27) | 12.0 (0–33) | n.s. | 6.6 (0–27) | 8.2 (0–33) | n.s. |

*Note:* Mean values are shown ±SD in case of normal distribution; otherwise, range is indicated in parentheses.
Abbreviations: BM, basement membrane; n.s., not significant.

**TABLE 3** Ultrastructural parameters of capillaries within subsets of auto-immune myositis controls in pooled cohorts

| Features | DM $n = 10$ | IBM $n = 13$ | IMNM $n = 11$ | ASS $n = 9$ | *p*-value | Subgroup analysis *p*-value |
|---|---|---|---|---|---|---|
| % of capillaries with 4+ layers BM reduplication | 7 (0–21) | 13 (0–33) | 3 (0–18) | 7 (0–18) | n.s. | n.s. (IBM vs others) |
| Mean number of BM layers per capillary | 2.0 (1.1–2.9) | 2.2 (1.3–3.0) | 1.6 (1.0–2.6) | 2.2 (1.0–4.0) | n.s. | |
| Maximum number of BM layers per capillary | 3.7 (2–7) | 4.2 (2–7) | 3.2 (1–4) | 3.6 (2–5) | n.s. | |
| % of capillaries with thick BM (>300 nm) without fine layering | 5 (0–33) | 33 (0–88) | 49 (0–100) | 21 (0–92) | **0.02** | **0.05 (IMNM vs others)** |
| Mean number of ensheathed pericyte processes per capillary | 2.8 (0.8–6.0) | 2.8 (1.3–4.1) | 2.4 (0.7–3.5) | 1.9 (1.2–5.1) | n.s. | |
| % of capillaries with endothelial activation | 80 (50–100) | 69 (21–100) | 52 (17–100) | 72 (28–100) | **0.046** | |
| Luminal diameter (μm) | 2.9 ± 0.9 | 2.3 ± 0.6 | 2.0 ± 0.6 | 2.2 ± 0.6 | **0.043** | |
| External diameter (μm) | 4.7 ± 1.2 | 3.8 ± 0.5 | 3.4 ± 0.6 | 3.7 ± 0.8 | **0.005** | **0.002 (DM vs others)** |
| % of dilated capillaries | 4.7 (0–33) | 0.5 (0–5) | 0.9 (0–5) | 3.9 (0–15) | n.s. | |
| % of collapsed capillaries | 10.0 (0–33) | 6.0 (0–28) | 9.0 (0–33) | 7.8 (0–26) | n.s. | |

*Note*: Mean values are shown ±SD in case of normal distribution; otherwise, range is indicated in parentheses.
Abbreviations: ASS, anti-synthetase syndrome; BM, basement membrane; DM, dermatomyositis; IBM, inclusion body myositis; IMNM, immune-mediated necrotizing myopathy; n.s., not significant.

proportion of dilated capillaries in SM cases compared with controls (5.2% vs 2.3%, $p = 0.011$). Detailed ultrastructural data from all cases and controls, including luminal and external diameters, presence of endothelial tubuloreticular inclusions (seen in 5 of 60 SM cases) and selected clinical data are included in Table S2A-B.

Among AIM controls, 4+ layers BM reduplication similar to SM was most often seen in IBM, affecting up to 20%–30% of capillaries in 5 of 13 cases (Table S2B), although this was not statistically different when compared with other AIM control subgroups (Table 3). Capillaries with diffuse thickening of BM without fine layering were more frequent in IMNM ($p = 0.05$) and capillaries showed larger mean external diameters in DM ($p = 0.002$).

## Capillary dilation is the most distinctive ultrastructural feature of SM cases without prominent BM reduplication

To better understand SM cases without prominent BM reduplication, we compared this subgroup with the controls and these SM had more frequent dilated capillaries (8% vs 2%, $p = 0.001$) and a larger number of ensheathed pericyte processes per capillary (3.4 vs 2.7, $p = 0.008$). In a ROC analysis of capillary ultrastructural parameters to predict SM among cases without prominent BM reduplication, the proportion of dilated capillaries had the most left-skewed curve (maximising specificity) (Figure 3B), and an optimal cut-off value of 2% offering a sensibility of 67% and specificity of 77%. Comparing SM cases with and without prominent BM reduplication, dilated capillaries were numerically more frequent in cases without this feature (8% vs 4%) although this failed to reach statistical significance ($p = 0.063$). There was no correlation between the proportion of dilated capillaries and disease duration among SM cases ($r = 0.081$, $p =$ n.s.).

## Capillary enlargement detected by Col4IF on light microscopy discriminates between SM cases and AIM controls

All detailed light microscopy data are presented in Table S2A-B. On routine haematoxylin-eosin-saffron (HES) stains, capillary enlargement was seen in 20 of 58 (35%) SM cases (only one of which with pipestem capillaries), and in 4 of 43 (9%) AIM controls ($p = 0.003$). Because evaluation of capillaries on routine stains is suboptimal, different stains including endothelial marker CD31 and BM marker Col4 were compared on either peroxidase immunohistochemistry or IF and Col4IF was selected for further capillary analysis because of its better contrast at low magnification (Figure 4A–D). Blinded review of Col4IF slides from cases and controls revealed capillary enlargement in 48 of the 57 (84%) SM cases and in 9 of the 43 (21%) AIM controls ($p < 0.001$) (Figure 4E–H). Capillary dropout was present in 32 of 57 (56%) SM cases and in 13 of 43 (30%) AIM controls ($p = 0.008$), mostly DM (7/13) and ASS (3/13). The Col4IF-stained BM of enlarged capillaries in SM often displayed a characteristic laminated appearance

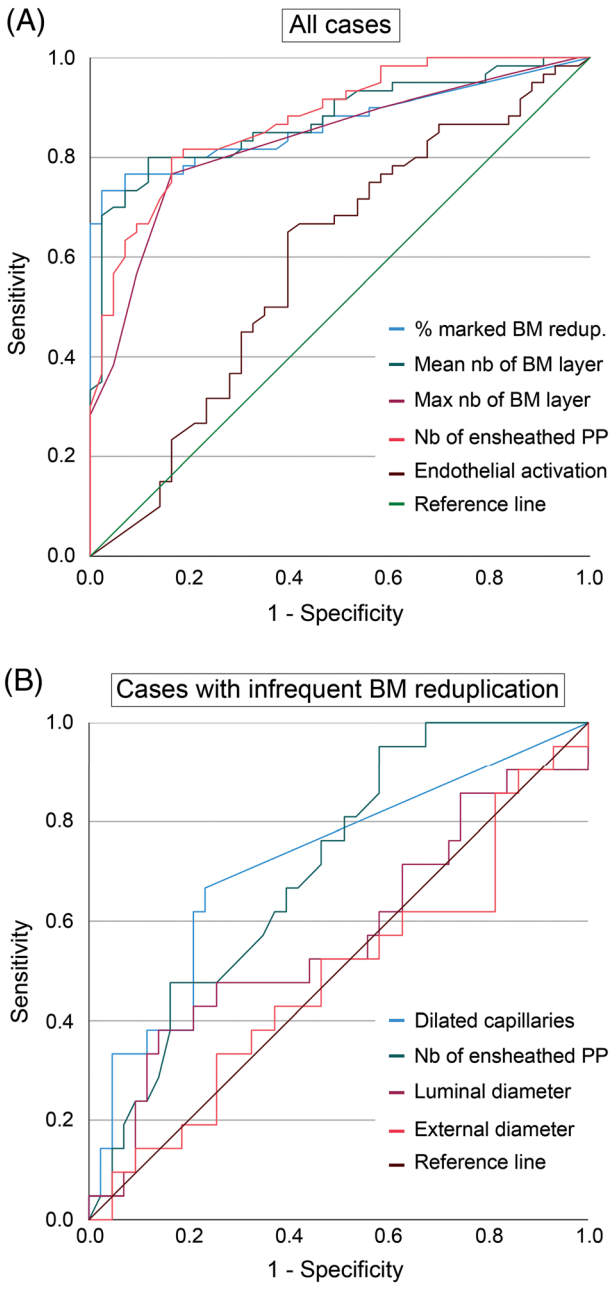


**FIGURE 3** ROC curves of ultrastructural parameters to detect SM from AIM controls of the discovery cohort. (A) all ultrastructural parameters except endothelial activation (AUC 0.661, 95% CI 0.496–0.827, $p = 0.060$) showed curves significantly different than the reference line with similar AUC (range 0.822–0.865, 95% CI 0.702–0.964, $p < 0.0001$) but the proportion of capillaries with 4+ layers BM reduplication was the parameter with the most left-skewed curve (maximising specificity) and the most practical cut-off value of 50% (offering a sensitivity of 65% and a specificity of 100%). (B) In the subset of cases without prominent BM reduplication, proportion of dilated capillaries and number of ensheathed PP per capillary showed curves significantly different than the reference line ($p = 0.005$ vs $p = 0.008$) and similar AUC (0.718 vs 0.705, 95% CI 0.578–0.858) but the proportion of dilated capillaries was the parameter with the optimal cut-off value (2%) closest to the (0,1) corner offering a sensibility of 67% and specificity of 77%. The optimal cut-off value for the number of ensheathed PP was 3, offering a sensitivity of 62% and specificity of 63%. AUC, area under the curve; AIM, auto-immune myositis; BM, basement membrane; capil, capillaries; nb, number; PP, pericyte processes; SM, scleromyositis

(Figure 4F). Capillary dropout without enlargement was more frequent in AIM controls than in SM cases (19% vs 4%, $p = 0.015$) and capillary enlargement without dropout was more frequent in SM cases than in controls (32% vs 9%, $p = 0.006$). When only relying on capillary enlargement on IF to detect SM cases, the sensitivity was 84% and specificity 79% (Table S3). When relying on both capillary enlargement and dropout, the sensitivity was 53% and the specificity 88%.

Ultrastructural measurements and IF data appeared consistent, as cases with capillary enlargement on IF showed significantly higher mean external diameters on EM than those without (4.3 μm vs 3.6 μm, $p < 0.001$). Cases with capillary enlargement on IF also showed higher proportions of dilated capillaries on EM (5.8% vs 1.6%, $p = 0.0145$), suggesting that some enlarged capillaries on IF were probably also dilated. Among SM cases without prominent BM reduplication, capillary enlargement on IF was more frequent in SM compared with controls (80% vs 21%, $p < 0.001$), but these may reflect dilated capillaries, already shown above to be a frequent finding in this subgroup of SM. When relying on either capillary enlargement on IF or on prominent BM reduplication on EM to detect SM from other AIMs, the sensitivity was 93% and the specificity 79% (Table S3).

## Light microscopy shows histomorphological heterogeneity of SM

Using only the adapted histopathological ENMC criteria, SM muscle biopsies showed heterogeneity and were classified either as non-inflammatory myopathy (NIM) (21/58, 36%), non-specific myositis (NSM) (19/58, 33%) or IMNM (18/58, 31%). To better describe the spectrum of features present, further histomorphological and immunohistochemical parameters were recorded and cases were subgrouped according to previously reported descriptive patterns (Tables S4A and S4B) [5,11–14]. Cases classified as NIM did not show lymphocytic infiltrates and either displayed very minimal pathology (Figure 5A), or more important fibre size variation and prominent endomysial fibrosis (Figure 5E). Cases classified as NSM showed even more heterogeneity, some having only mild perivascular lymphocytic infiltrates and others dense perimysial infiltrates often rich in plasma cells and focally extending into the endomysium (Figure 5I). Fibre size variation was mild to severe, as was the degree of necrosis. Cases classified as IMNM all had scattered necrotic fibres without endomysial lymphocytic infiltrates (Figure 5M), but some did show focal perivascular infiltrates slightly in excess of what is usually seen in IMNM, whereas others showed significant endomysial fibrosis and

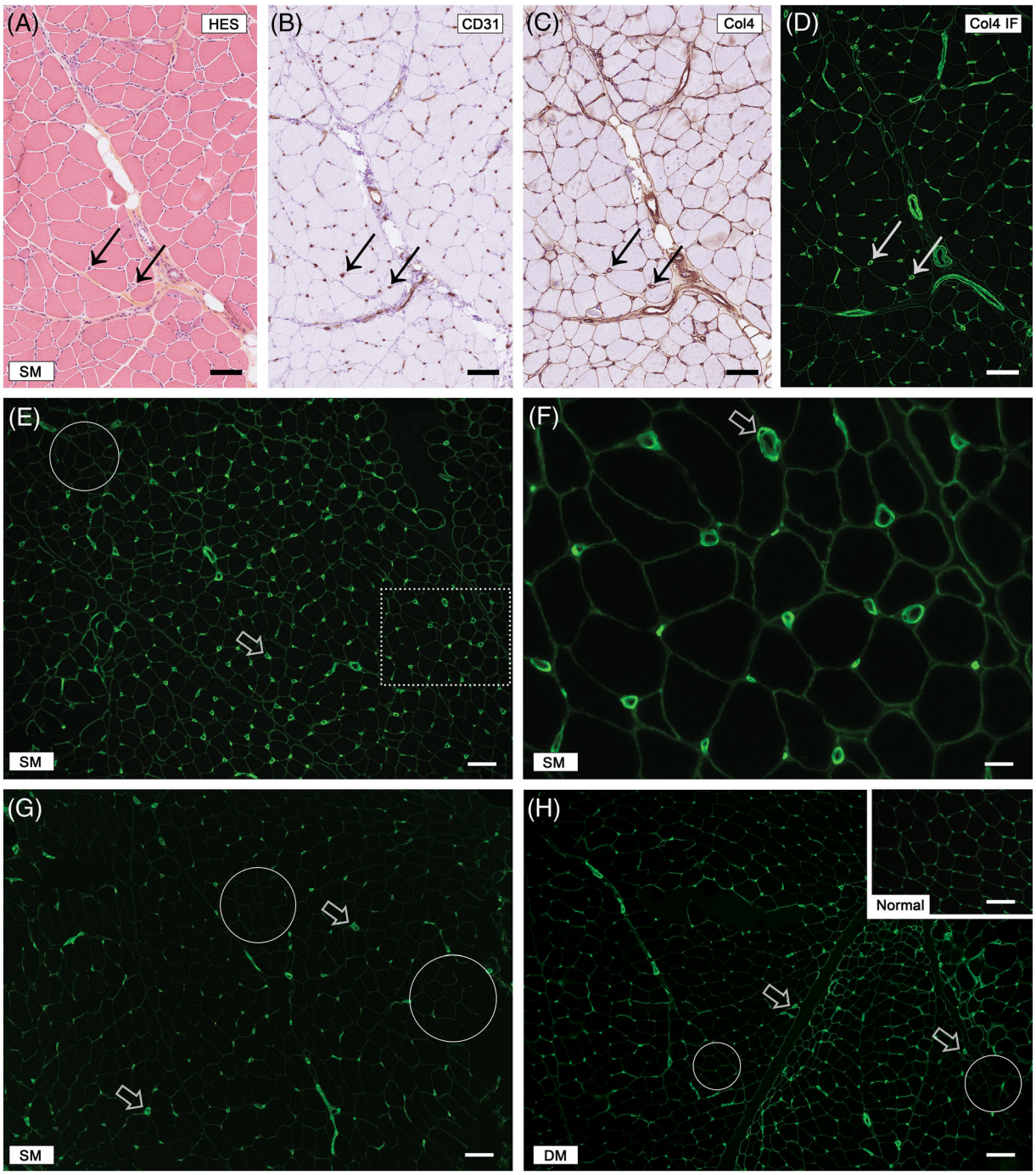


**FIGURE 4** Light microscopic features of endomysial capillaries in SM and AIM controls. (A–D) Patient #5. Comparison of HES routine stain (A), immunohistochemistry for endothelial cell maker CD31 (B) or BM marker Col4 (C) and IF for Col4 (D) reveals better low magnification contrast with Col4IF to highlight enlarged capillaries (arrows). (E–G) Col4IF highlighting capillaries in SM and controls. (E) Patient #7. Capillary dropout (circled area) and enlargement (open arrow) in a representative case of SM with prominent BM reduplication. (F) Higher magnification of dotted area in (E) to show laminated appearance of BM (open arrow). (G) Patient #9. Capillary dropout (circled areas) with only focal capillary enlargement (open arrows) in a case of SM with prominent BM reduplication classified on light microscopy as non-inflammatory myopathy (minimal myositis with capillary pathology). (H) Control #27. Capillary dropout (circled areas) with focal capillary enlargement (open arrows) in a representative case of dermatomyositis. Inset in (H), Normal control with normal capillary size and capillary density. All bars, 100 μm, except (F), 25 μm. AIM, auto-immune myositis; BM, basement membrane; Col4, collagen type 4; HES, haematoxylin-eosin-saffron; IF, immunofluorescence; IMNM, immune-mediated necrotizing myopathy; SM, scleromyositis

marked fibre size variation as can be seen in IMNM with dystrophic pathology. Sarcolemmal expression of MHC1 was present in 50 of 58 cases (86%), more strongly in cases classified as NSM where it was often associated with MHC2 expression, but also in many of the cases classified as NIM or IMNM. The pattern of MHC1 and MHC2 expression was either perifascicular (Figure 5K,L) or focal (Figure 5O,P). Sarcolemmal deposition of C5b-9 was seen in 27 of 58 cases (47%), sometimes in a perifascicular pattern (Figure 5J), sometimes only focally (Figure 5N). No case showed sarcoplasmic MxA expression (only analysed in validation cohort). Denervation changes such as fibre-type grouping or clusters of atrophic angulated fibres were only rarely seen.

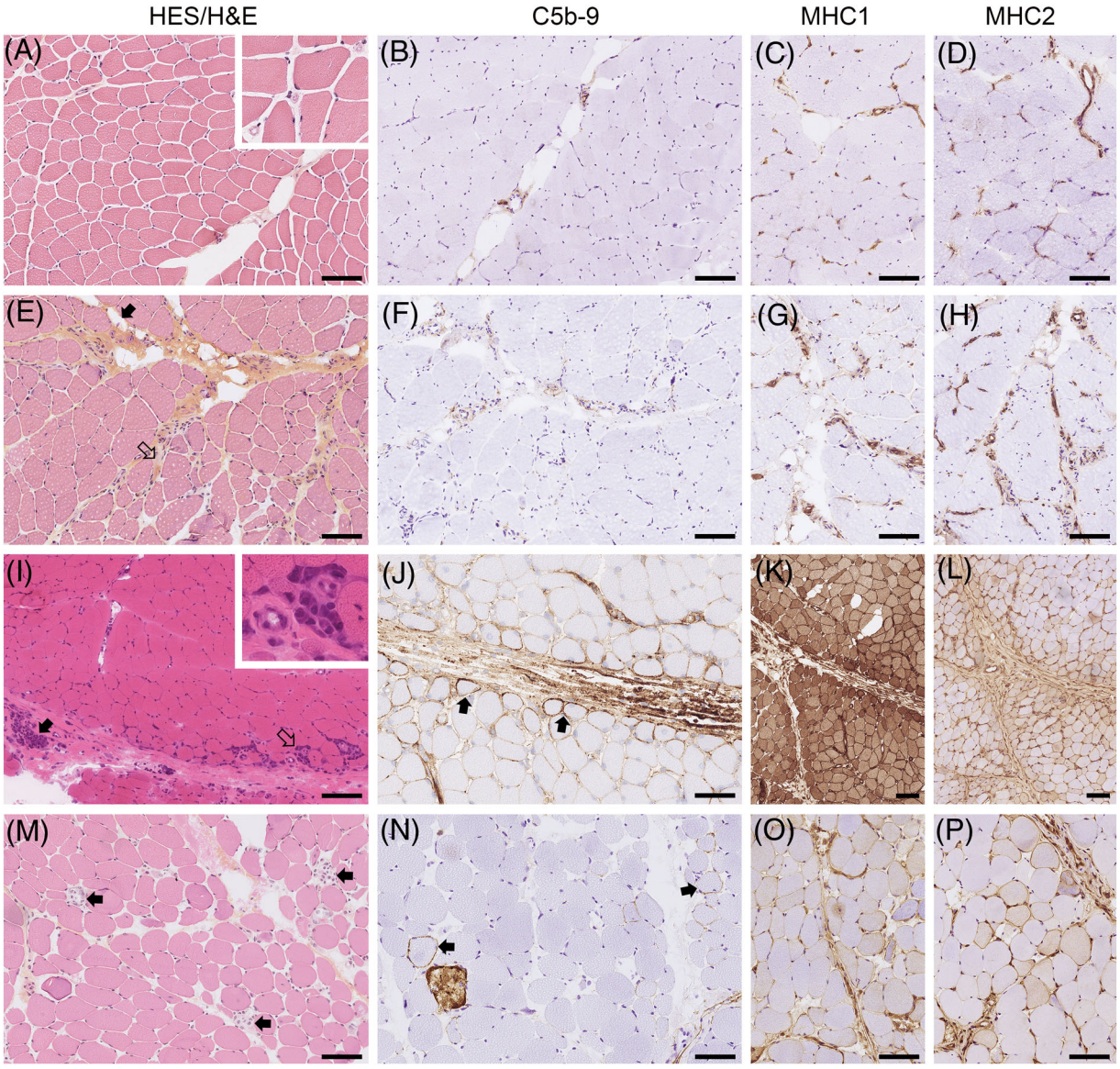


**FIGURE 5** Histomorphological patterns and immunohistochemical profiles encountered in SM patients. (A–D) Patient #9. Non-inflammatory myopathy (MMCP pattern) with few split or atrophic fibres (A, HES) and dilated and enlarged capillaries (inset), no C5b-9 deposition (B), and no sarcolemmal MHC1 (C) or MHC2 (D) expression. (E–H) Patient #23. Non-inflammatory myopathy (fibrotic pattern) with marked variation in fibre size, endomysial fibrosis (open arrow) and mild fatty change (E, HES, solid arrow), with no sarcolemmal C5b-9 deposition (F), and no sarcolemmal MHC1 (G) or MHC2 expression (H). (I–L) Patient #52. Non-specific myositis (inflammatory pattern) with perimysial mononuclear inflammatory infiltrates (solid arrow) rich in plasma cells and extending into adjacent endomysium (open arrow and inset) (I, H&E), frequent perifascicular fibres with sarcolemmal C5b-9 deposition (arrows) (J), and perifascicular sarcolemmal expression of MHC1 (K) and MHC2 (L). (M–P) Patient #11. IMNM-like pattern with frequent randomly distributed necrotic fibres (arrows) without lymphocytic infiltration (M, HES), scattered non-necrotic fibres with sarcolemmal C5b-9 deposition (arrows) (N), and moderate sarcolemmal MHC1 (O) and MHC2 (P) expression. All bars 100 μm, except (B), 50 μm. ENMC, European NeuroMuscular centre; HES, haematoxylin-eosin-saffron; IMNM, immune-mediated necrotizing myopathy; MMCP, minimal myositis with capillary pathology; SM, scleromyositis

### Capillary pathology is consistently encountered through the entire clinico-sero-pathological spectrum of SM

The proportion of capillaries with 4+ layers BM reduplication was similar between SM cases classified as NIM, NSM or IMNM ($p$ = n.s.) (Figure S3A) or between SM of different histomorphological patterns ($p$ = n.s.) (Figure S3B), as were the proportions of dilated or thick unlayered capillaries ($p$ = n.s.). Patients with grade 2–3 endomysial fibrosis (see Table S4A) did not have significantly different disease duration ($p$ = n.s.).

SM patients with or without prominent BM reduplication did not show significant differences in duration of disease, CK levels, serological categories or the following vasculopathic features: Raynaud's phenomenon, digital ulcers, fingertip pitting scars, abnormal nailfold capillaroscopy, pulmonary arterial hypertension or scleroderma renal crisis ($p$ = n.s.). These clinico-serological features were also compared between SM patients with and without an IMNM-like ENMC pattern and no significant differences were revealed ($p$ = n.s.) except for higher CK levels in the IMNM-like subset (3730 vs 1427 IU/L, $p < 0.001$).

In the subset of SM cases without prominent BM reduplication, 15 of 21 (71%) patients fulfilled the ACR/EULAR SSc criteria, 18 of 21 (86%) had Raynaud's phenomenon and 15 of 19 (71%) had SSc-type capillaroscopy. In the subset of SM cases sine scleroderma, 16 of 24 (67%) showed prominent BM reduplication and in those who did not fulfil the ACR/EULAR SSc criteria, 10 of 16 (63%) showed this feature. Cases in this subset did not have a higher proportion of dilated capillaries on EM or of enlarged capillaries on IF ($p$ = n.s.). In the subset of patients who were seronegative for known SSc autoantibodies, 16 of 24 (67%) showed prominent BM reduplication.

## DISCUSSION

Herein, we provide the largest ultrastructural and light microscopy comparison of endomysial capillaries between SM and the main currently recognised AIM subsets with validation in an independent cohort. Our results show that prominent capillary BM reduplication (4+ layers in most capillaries) is a distinct finding of SM. Indeed, 65% of our SM cases showed prominent BM reduplication compared with none of the controls. Many of the 43 controls did have capillaries with 4+ layers BM reduplication, but this generally represented <20% of capillaries and only rarely up to 33%, mostly among IBM controls. Legacy morphometric studies had reported 66%–74% of endomysial capillaries with BM reduplication in a series of 5 SSc patients, 24%–62% of capillaries in 15 patients with other myositis (DM, polymyositis, lupus) and up to 66% of capillaries in 25 patients with Duchenne and other muscular dystrophies, but a distinction was not made between capillaries with only two to three layers of reduplicated BM and capillaries with 4+ layers [26,27], which our data suggest is a more specific finding of SM.

Siegert et al recently reported the presence of capillaries with 4+ layers BM reduplication in 61% of 18 cases of SSc-associated myositis as part of a set of ultrastructural capillary changes also including BM thickening, endothelial activation and ensheathment of pericyte processes [14]. Our data also show that increased numbers of ensheathed pericyte processes is characteristic of SM, although slightly less specific than prominent BM reduplication in our group of 43 controls. Although total BM thickness should increase proportionally with the number of reduplicated BM layers, thickness measurements alone would not be sufficient to differentiate SM from controls because in our cohort, the proportion of capillaries with diffuse thickening of BM without fine layering (as also often seen in type 2 diabetes [28]) was actually higher in controls. In our series, endothelial activation did not discriminate between SM and controls.

The exact pathophysiology of capillary BM reduplication is not known but a similar change has been observed in experimentally induced capillary necrosis by cold or ischaemia [29]; therefore, BM reduplication in SM may conceivably represent repeated cycles of capillary degeneration and regeneration. Our data revealed that in SM, the number of reduplicated BM layers correlates closely with the number of ensheathed pericyte processes suggesting that the two are pathophysiologically related. Because pericytes are contractile cells that help regulate blood flow by varying capillary diameter [30], pericyte dysfunction could conceivably cause capillary dilation as an early event in SM, reminiscent of megacapillaries seen on nailfold capillaroscopy of SSc patients, followed by stages of gradual capillary contraction and degeneration leaving behind a trail of concentric reduplicated BM layers. Studies have shown the potential role of pericyte dysfunction in SSc including via a proposed pericyte-to-myofibroblast transition leading to tissue fibrosis [30].

On routine light microscopy, capillary changes are often inconspicuous and differentiating endothelial and basement membrane changes is a challenge. We used Col4IF to stain the BM of capillaries and found that in SM cases it often showed a laminated appearance, consistent with BM reduplication rather than diffuse BM thickening. Col4IF also revealed that cases with enlarged capillaries were ~4 times more frequent in SM than in controls. However, because Col4IF only stains the BM surrounding the endothelial cell, the relative proportion of lumen to endothelial cell in the central unstained area of each capillary on Col4IF cannot be determined and capillaries that appear large may actually be thin walled and dilated. Our ultrastructural analysis reliably differentiated between dilation and enlargement and revealed significantly more frequent dilated capillaries in SM than in controls, a finding consistent with the frequent observations of large thin-walled capillaries in SM in some of the legacy morphometric studies [18,31]. In our SM cases without prominent BM reduplication, dilated capillaries even appeared to be the most specific parameter to help discriminate SM cases from controls.

We also used Col4IF for capillary dropout assessment because of its better contrast than CD31 immunohistochemistry at low magnification. Our Col4IF data showed that capillary dropout was more frequent in SM than in controls but suggest that capillary enlargement/

dilation may be an earlier event than dropout in SM because many of our cases showed enlargement without dropout, but dropout was rarely seen alone. This hypothesis is also supported by nailfold capillaroscopy studies which typically show capillary enlargement before capillary loss in SSc patients [32]. On the other hand, perhaps due to the relatively small number of patients, our data failed to reveal shorter disease duration or higher proportion of cases not fulfilling ACR/EULAR SSc criteria in the subset of patients with capillary enlargement on Col4IF or with more frequent capillary dilation on EM.

Vascular injury is central to both DM and SM pathophysiology but the underlying mechanisms most likely differ [14,16,33]. Capillary dropout is frequent in both, but in DM, it is generally found along with perifascicular atrophy, a finding we did not see in our SM cases. Endothelial tubuloreticular inclusions and MxA overexpression are biomarkers of type 1 interferon signalling activation [34] and are considered the ultrastructural and immunohistochemical hallmarks of DM [22]. They are present in 71% of cases [35] and support DM as a distinct subset despite its heterogeneity on light microscopy [8]. Likewise, prominent BM reduplication was present in 65% of our SM cases, in similar proportions across patterns that included non-specific inflammatory [11], IMNM-like [12], minimal myositis with capillary pathology [14] and fibrosing myopathy [12,13], bringing support to the concept of SM as a distinct AIM subset despite its heterogeneity on light microscopy.

Despite shared involvement of capillaries, it is important to emphasise that the challenge for the pathologist is not to differentiate SM from DM, but rather to recognise SM in muscle biopsies that appear non-specific or that mimic IMNM. In patients negative for anti-SRP or anti-HMGCR autoantibodies, failure to recognise SM on muscle biopsy with isolated necrotic fibres and little inflammation may lead to overdiagnosis of IMNM and delay appropriate screening for SSc cardiopulmonary involvement [10] and potentially increase the risk of corticosteroid-induced scleroderma renal crisis [36]. As recently observed by Allenbach et al in the differential diagnosis of IMNM [37], we found that perifascicular MHC1/MHC2 expression and perivascular lymphocytic infiltration in excess of what is usually seen in IMNM can be used as red flags for SM. However, some cases show no clues to distinguish SM from IMNM on routine stains and further examination of capillaries by Col4IF and EM are critical to make the appropriate diagnosis. Our results suggest that patients with non-specific myositis, seronegative IMNM, or with clinico-serological suspicion of SM and non-inflammatory or IMNM-like biopsies are ideal candidates for capillary pathology evaluation by Col4IF and, for higher specificity, by EM.

Failure to recognise SM is also more likely to happen in patients with early/mild SSc presenting sine scleroderma most not fulfilling the ACR/EULAR SSc criteria [7]. Many SSc patients presenting initially with myositis may take years to fulfil these criteria and our cohorts are the first to include them in an ultrastructural study. Indeed, 40% of our SM patients presented sine scleroderma among which 54% did not fulfil the ACR/EULAR SSc criteria and had shorter disease duration. Yet, most of them showed prominent BM reduplication. This form of microvascular damage, similar to that seen on EM of skin in both early [38] and established [39] SSc, and the strong association with Raynaud's phenomenon, support the concept of myositis as an organ manifestation of SSc rather than an associated disease. Indeed, microvascular damage is a well-established predictor of Raynaud's progression to SSc [32].

There is a growing interest in SM and current literature suggests its prevalence has often been underestimated [1]. This may be due to the fact that SM may remain clinically undetected, as shown in a recent MRI study that demonstrated high proportion of subclinical disease [40]. Our data add to this expanding body of work and have the potential to improve outcomes in SM. Importantly, because microvascular damage appears to be central in the pathophysiology of SM, novel approaches targeting vasculopathy could conceivably complement our therapeutic arsenal beyond conventional immunosuppression.

In summary, we showed in muscle biopsies from a large number of SM patients, including many with mild SSc features or early disease, that 4+ layers capillary BM reduplication is a frequent ultrastructural finding, whereas it is only seen in a minority of capillaries in other myositis subsets. Our findings show that EM is an essential tool for the specific and early recognition of SM and support the concept of SM as an organ manifestation of SSc and a distinct subset of AIM.

## ACKNOWLEDGMENTS

The authors would like to thank Carl Faille, Émilie Fréreault and Hélène Tanguay at CHU Sainte-Justine, Division of Pathology, for histotechnical expertise, Dre Marie-Pierre Fournier-Gosselin at the Centre hospitalier de l'Université de Montréal, Division of Neurosurgery, for performing muscle biopsies and Mianbo Wang at the Jewish General Hospital for statistical support.

## CONFLICT OF INTERESTS

None of the authors have conflict of interests to disclose.

## ETHICS STATEMENT

All SM patients enrolled provided written informed consent. Control biopsies were from archival cases and used with proper authorization. Ethics approval for this study was obtained at the Jewish General Hospital and CHU Sainte-Justine, Montreal, Canada.

## AUTHOR CONTRIBUTION

Conception, design, analysis and interpretation of data: BE, VL, YT, JLS, MH, AM and OLC. Critical revision of the manuscript for important intellectual content: BE, VL, YT, IB, MG, SH, JBT, VN, JD, JK, EOF, BL, MS, MJF, JLS, MH, AM and OLC.

## DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article

## ORCID

*Benjamin Ellezam* https://orcid.org/0000-0002-8716-7924

*Océane Landon-Cardinal* https://orcid.org/0000-0002-3361-6165


## REFERENCES


1. Walker UA, Clements PJ, Allanore Y, et al. Muscle involvement in systemic sclerosis: points to consider in clinical trials. *Rheumatology (Oxford)*. 2017;56(suppl_5):v38-v44. doi:10.1093/rheumatology/kex196
2. Johnson SR, Fransen J, Khanna D, et al. Validation of potential classification criteria for systemic sclerosis. *Arthritis Care Res (Hoboken)*. 2012;64(3):358-367. doi:10.1002/acr.20684
3. van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/-European league against rheumatism collaborative initiative. *Ann Rheum Dis*. 2013;72(11):1747-1755. doi:10.1136/annrheumdis-2013-204424
4. Lundberg IE, Tjärnlund 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. *Ann Rheum Dis*. 2017;76(12):1955-1964. doi:10.1136/annrheumdis-2017-211468
5. Bhansing KJ, Lammens M, Knaapen HKA, van Riel PLCM, van Engelen BGM, Vonk MC. Scleroderma-polymyositis overlap syndrome versus idiopathic polymyositis and systemic sclerosis: a descriptive study on clinical features and myopathology. *Arthritis Res Ther*. 2014;16(3):R111. doi:10.1186/ar4562
6. Meyer A, Leclair V, Landon-Cardinal O, et al. ACR/EULAR criteria for myositis and systemic sclerosis lack sensitivity for Scleromyositis [abstract]. *Arthritis Rheumatol*. 2019;71(suppl 10):629.
7. Landon-Cardinal O, Baril-Dionne A, Hoa S, et al. Recognising the spectrum of scleromyositis: HEp-2 ANA patterns allow identification of a novel clinical subset with anti-SMN autoantibodies. *RMD Open*. 2020;6(2):e001357. doi:10.1136/rmdopen-2020-001357
8. Allenbach Y, Benveniste O, Goebel HH, Stenzel W. Integrated classification of inflammatory myopathies. *Neuropathol Appl Neurobiol*. 2017;43(1):62-81. doi:10.1111/nan.12380
9. Hoogendijk JE, Amato AA, Lecky BR, et al. 119th ENMC international workshop: trial design in adult idiopathic inflammatory myopathies, with the exception of inclusion body myositis, 10–12 October 2003, Naarden, The Netherlands. *Neuromuscul Disord*. 2004;14(5):337-345. doi:10.1016/j.nmd.2004.02.006
10. Paik JJ. Muscle disease in scleroderma. *Curr Opin Rheumatol*. 2018;30(6):576-580. doi:10.1097/BOR.0000000000000552
11. Ranque B, Authier FJ, Le-Guern V, et al. A descriptive and prognostic study of systemic sclerosis-associated myopathies. *Ann Rheum Dis*. 2009;68(9):1474-1477. doi:10.1136/ard.2008.095919
12. Paik JJ, Wigley FM, Lloyd TE, et al. Spectrum of muscle histopathologic findings in forty-two scleroderma patients with weakness. *Arthritis Care Res (Hoboken)*. 2015;67(10):1416-1425. doi:10.1002/acr.22620
13. Paik JJ, Wigley FM, Shah AA, et al. Association of Fibrosing Myopathy in systemic sclerosis and higher mortality. *Arthritis Care Res (Hoboken)*. 2017;69(11):1764-1770. doi:10.1002/acr.23291
14. Siegert E, Uruha A, Goebel H-H, et al. Systemic sclerosis-associated myositis features minimal inflammation and characteristic capillary pathology. *Acta Neuropathol*. 2021;141(6):917-927. doi:10.1007/s00401-021-02305-3
15. Fleischmajer R, Perlish JS. Capillary alterations in scleroderma. *J am Acad Dermatol*. 1980;2(2):161-170. doi:10.1016/S0190-9622(80)80396-3
16. Corallo C, Cutolo M, Volpi N, et al. Histopathological findings in systemic sclerosis-related myopathy: fibrosis and microangiopathy with lack of cellular inflammation. *Ther Adv Musculoskelet Dis*. 2017;9(1):3-10. doi:10.1177/1759720X16671928
17. Michalowski R, Kudejko J. Electron microscopic observations on skeletal muscle in diffuse scleroderma. *Br J Dermatol*. 1966;78(1):24-28. doi:10.1111/j.1365-2133.1966.tb12129.x
18. Norton WL, Hurd ER, Lewis DC, Ziff M. Evidence of microvascular injury in scleroderma and systemic lupus erythematosus: quantitative study of the microvascular bed. *J Lab Clin Med*. 1968;71(6):919-933.
19. Thompson JM, Bluestone R, Bywaters EG, Dorling J, Johnson M. Skeletal muscle involvement in systemic sclerosis. *Ann Rheum Dis*. 1969;28(3):281-288. doi:10.1136/ard.28.3.281
20. Ellezam B, Leclair V, Troyanov Y, Meyer A, Hudson M, Landon-Cardinal O. Capillary basement membrane reduplication in myositis patients with mild clinical features of systemic sclerosis supports the concept of 'scleromyositis'. *Acta Neuropathol*. 2021;142(2):395-397. doi:10.1007/s00401-021-02335-x
21. Poormoghim H, Lucas M, Fertig N, Medsger TA Jr. Systemic sclerosis sine scleroderma: demographic, clinical, and serologic features and survival in forty-eight patients. *Arthritis Rheum*. 2000;43(2):444-451. doi:10.1002/1529-0131(200002)43:2%3C444::AID-ANR27%3E3.0.CO;2-G
22. Mammen AL, Allenbach Y, Stenzel W, Benveniste O, Group EtWS. 239th ENMC international workshop: classification of dermatomyositis, Amsterdam, the Netherlands, 14-16 December 2018. *Neuromuscul Disord*. 2020;30(1):70-92. doi:10.1016/j.nmd.2019.10.005
23. Allenbach Y, Mammen AL, Benveniste O, et al. 224th ENMC international workshop: Clinico-sero-pathological classification of immune-mediated necrotizing myopathies. *Neuromuscul Disord*. 2018;28(1):87-99. doi:10.1016/j.nmd.2017.09.016
24. Lloyd TE, Mammen AL, Amato AA, Weiss MD, Needham M, Greenberg SA. Evaluation and construction of diagnostic criteria for inclusion body myositis. *Neurology*. 2014;83(5):426-433. doi:10.1212/WNL.0000000000000642
25. De Bleecker JL, De Paepe B, Aronica E, et al. 205th ENMC international workshop: pathology diagnosis of idiopathic inflammatory myopathies part II 28-30 march 2014, Naarden, The Netherlands. *Neuromuscul Disord*. 2015;25(3):268-272. doi:10.1016/j.nmd.2014.12.001
26. Jerusalem F, Rakusa M, Engel AG, MacDonald RD. Morphometric analysis of skeletal muscle capillary ultrastructure in inflammatory myopathies. *J Neurol Sci*. 1974;23(3):391-402. doi:10.1016/0022-510X(74)90157-9
27. Jerusalem F, Engel AG, Gomez MR, Duchenne dystrophy. I. Morphometric study of the muscle microvasculature. *Brain*. 1974;97(1):115-122. doi:10.1093/brain/97.1.115
28. Siperstein MD, Unger RH, Madison LL. Studies of muscle capillary basement membranes in normal subjects, diabetic, and prediabetic patients. *J Clin Invest*. 1968;47(9):1973-1999. doi:10.1172/JCI105886
29. Vracko R, Benditt EP. Basal lamina: the scaffold for orderly cell replacement. Observations on regeneration of injured skeletal muscle fibers and capillaries. *J Cell Biol*. 1972;55(2):406-419. doi:10.1083/jcb.55.2.406
30. Romano E, Rosa I, Fioretto BS, Matucci-Cerinic M, Manetti M. New insights into Profibrotic Myofibroblast formation in systemic sclerosis: when the Vascular Wall becomes the enemy. *Life (Basel)*. 2021;11(7):1-26. doi:10.3390/life11070610
31. Norton WL. Comparison of the microangiopathy of systemic lupus erythematosus, dermatomyositis, scleroderma, and diabetes mellitus. *Lab Invest*. 1970;22(4):301-308.
32. Koenig M, Joyal F, Fritzler MJ, et al. Autoantibodies and microvascular damage are independent predictive factors for the progression of Raynaud's phenomenon to systemic sclerosis: a twenty-year

prospective study of 586 patients, with validation of proposed criteria for early systemic sclerosis. *Arthritis Rheum*. 2008;58(12):3902-3912. doi:10.1002/art.24038
33. Ladislau L, Suárez-Calvet X, Toquet S, et al. JAK inhibitor improves type I interferon induced damage: proof of concept in dermatomyositis. *Brain*. 2018;141(6):1609-1621. doi:10.1093/brain/awy105
34. Greenberg SA, Pinkus JL, Pinkus GS, et al. Interferon-α/β-mediated innate immune mechanisms in dermatomyositis. *Ann Neurol*. 2005;57(5):664-678. doi:10.1002/ana.20464
35. Uruha A, Nishikawa A, Tsuburaya RS, et al. Sarcoplasmic MxA expression: a valuable marker of dermatomyositis. *Neurology*. 2017;88(5):493-500. doi:10.1212/WNL.0000000000003568
36. Steen VD, Medsger TA. Case-control study of corticosteroids and other drugs that either precipitate or protect from the development of scleroderma renal crisis. *Arthritis Rheum*. 1998;41(9):1613-1619. doi:10.1002/1529-0131(199809)41:9%3C1613::AID-ART11%3E3.0.CO;2-O
37. Allenbach Y, Benveniste O, Stenzel W, Boyer O. Immune-mediated necrotizing myopathy: clinical features and pathogenesis. *Nat Rev Rheumatol*. 2020;16(12):689-701. doi:10.1038/s41584-020-00515-9
38. Fleischmajer R, Perlish JS, Shaw KV, Pirozzi DJ. Skin capillary changes in early systemic scleroderma. Electron microscopy and "in vitro" autoradiography with tritiated thymidine. *Arch Dermatol*. 1976;112(11):1553-1557. doi:10.1001/archderm.1976.01630350029007
39. Trotta F, Biagini G, Cenacchi G, et al. Microvascular changes in progressive systemic sclerosis: immunohistochemical and ultrastructural study. *Clin Exp Rheumatol*. 1984;2(3):209-215.
40. Ross L, Lindqvist A, Costello B, et al. Using magnetic resonance imaging to map the hidden burden of muscle involvement in systemic sclerosis. *Arthritis Res Ther*. 2022;24(1):84 doi:10.1186/s13075-022-02768-z

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Ellezam B, Leclair V, Troyanov Y, et al. Capillary pathology with prominent basement membrane reduplication is the hallmark histopathological feature of scleromyositis. *Neuropathol Appl Neurobiol*. 2022;e12840. doi:10.1111/nan.12840