

Muscle biopsy abnormalities in systemic lupus erythematosus: correlation with clinical and laboratory parameters

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Abstract

Objectives—To investigate the incidence and significance of Type II fibre atrophy, vessel wall thickening, lymphocytic vasculitis and myositis in needle quadriceps muscle biopsies from patients with systemic lupus erythematosus (SLE) and their correlations with clinical and laboratory parameters.

Methods—Needle quadriceps muscle biopsies from 55 patients with SLE and 26 controls were prospectively examined. Clinical and laboratory parameters recorded at the time of muscle biopsy included arthralgia, arthritis, myalgia, proximal weakness, vasculitic rashes, Schirmer test, ENA antibodies, ESR, serum creatine kinase (CK) and plasma C3 degradation products.

Results—Abnormal muscle biopsies were significantly more frequent in patients with SLE compared with controls ($P < 0.005$). None of the controls had lymphocytic vasculitis and/or myositis. The difference in incidence between patients with SLE and controls for lymphocytic vasculitis was significant at $P < 0.005$. Due to the small number of SLE patients with myositis, the difference in incidence for this abnormal finding reached only $P = 0.09$. In the SLE patient group, lymphocytic vasculitis was associated with significantly higher ESR values ($P = 0.007$) and higher incidence of arthritis ($P = 0.01$); and appears to characterise a subset of patients with positive Schirmer tests, anti-Ro and/or anti-La antibodies. Raised serum CK was found to correspond with underlying myositis in patients with SLE and these patients also had an increased incidence of symptoms of proximal weakness and/or anti-RNP antibodies. In contrast, both Type II fibre atrophy and vessel wall thickening failed to correlate with any of the clinical and laboratory parameters studied and appear to be non-specific findings.

Conclusions—Abnormal muscle biopsies are common in patients with SLE and the presence of lymphocytic vasculitis and/or myositis signify pathology in these patients. Histopathological abnormalities in needle quadriceps muscle biopsies are further valuable parameters in the assessment of patients with SLE.

Systemic lupus erythematosus (SLE) is a multi-system chronic inflammatory disease with a wide spectrum of clinical and serological manifestations.¹ Assessment of disease activity in patients with SLE is predominantly clinical, assisted by urinalysis, measurement of erythrocyte sedimentation rate (ESR), serum/plasma complement levels and anti-double-stranded DNA (dsDNA) antibodies; and less commonly, renal biopsy.

Clinical evidence of skeletal muscle involvement is not uncommon in patients with SLE and is shown as weakness, myalgia and atrophy, often in a proximal distribution.^{2,3} It is not peculiar to SLE, however, being present in other connective tissue and metabolic disorders. Reported biopsy and necropsy series describe a histological spectrum of abnormal findings in muscle of patients with SLE with particular emphasis on the presence of myositis; myositis accounts for only a 5–11% of muscle changes in SLE.⁴ A systemic prospective pathological assessment of changes in muscle biopsy specimens in patients with SLE compared with controls is lacking, and the relationship between these abnormalities and the clinical status of the patient has not been fully addressed.

Histopathological changes described in the muscle of patients with SLE include the following findings: (1) myositis; (2) vasculitis; (3) type II fibre atrophy; (4) vessel wall thickening; (5) vacuolar myopathy and (5) neurogenic muscle injury. This prospective study investigates the incidence and significance of type II fibre atrophy, vessel wall thickening, lymphocytic vasculitis and myositis in needle quadriceps muscle biopsies from patients with SLE and their correlations with clinical and laboratory parameters. These four abnormalities were chosen as they are most frequently seen in patients with SLE. Furthermore, both lymphocytic vasculitis and myositis are acute and potentially reversible muscle changes and thus could be valuable indicators of disease activity with consequent implications for therapy.⁵

Patients and methods

PATIENTS

Needle quadriceps muscle biopsies from 55 patients with SLE, fulfilling the 1982 revised ARA criteria,⁶ were prospectively examined. All patients were attending the connective tissue disease clinic at Queens Medical Centre, a secondary referral centre and fall into two

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groups: (1) consecutive newly presenting patients; and (2) patients with clinically suspected flare up of their disease from whom muscle biopsies were taken as part of their disease assessment. Control quadriceps muscle

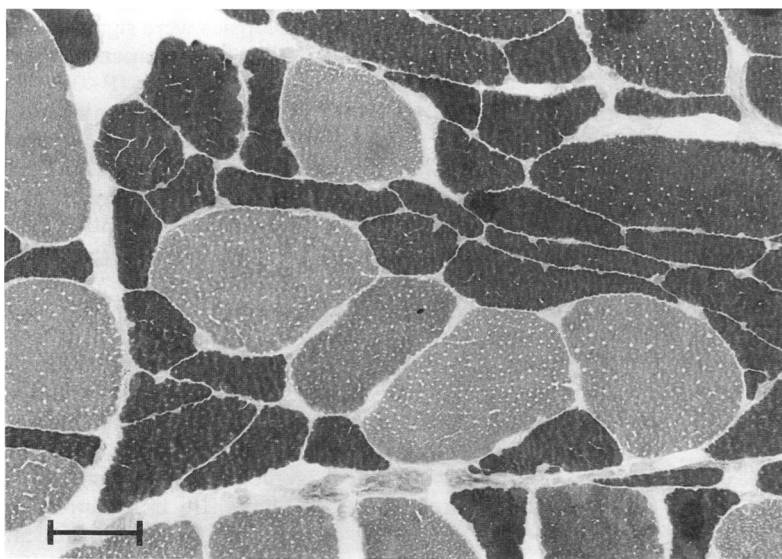


Figure 1 ATPase pH 9.4 showing atrophy of type II (dark) fibres. Bar = 50 μ m.

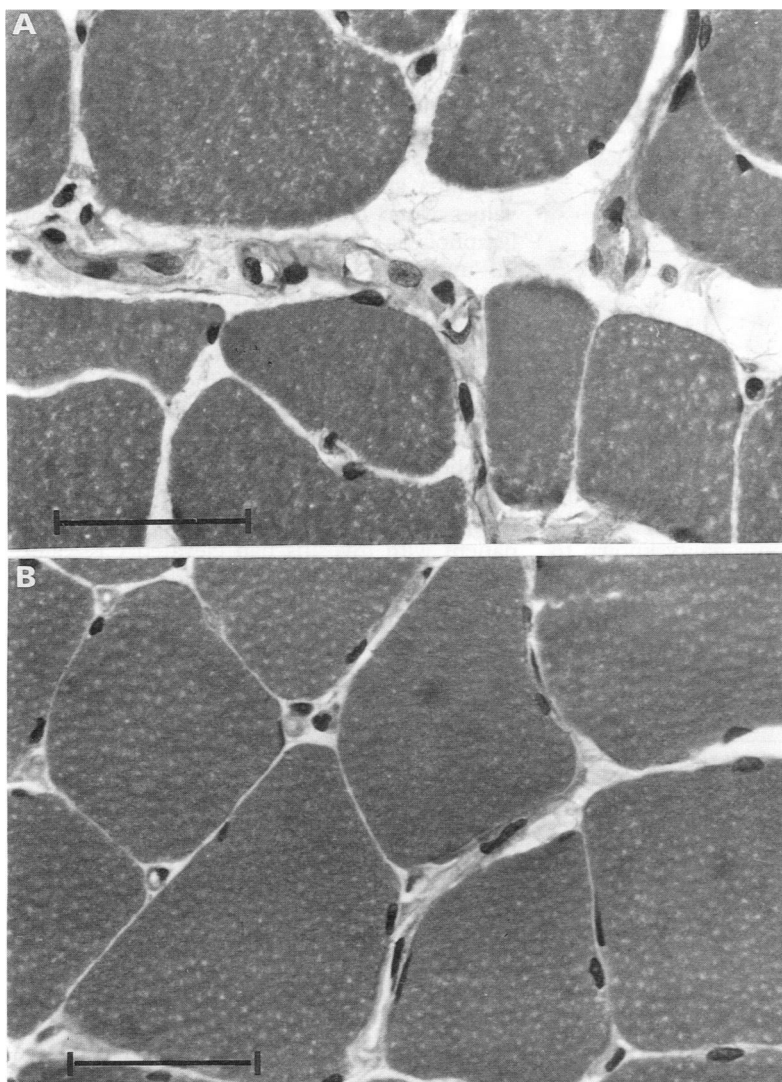


Figure 2 Thickening of small vessel walls (A) compared with normal vessels (B). Bar = 50 μ m.

tissues were obtained from 26 patients referred with non-specific symptoms, including arthralgia and myalgia. Symptoms in 17 of the 26 'control patients' were considered to be related to the chronic fatigue syndrome/fibromyalgia.⁷ These patients were included as controls as previous studies, using histochemical and immunoenzymatic techniques, have documented normal muscle biopsies on light microscopy in such patients.⁸ The rest of the controls (9 patients) were found to have no significant medical conditions. Consent was obtained from all patients before outpatient muscle biopsy. Approval for this study was given by the Nottingham University Ethics Committee.

At the time of muscle biopsy, the following were recorded in the patients with SLE: (1) drug history; (2) time of muscle biopsy in relation to first manifestation of disease; (3) presence of clinical disease features (arthralgia, arthritis, myalgia, proximal weakness and vasculitic rashes); (4) Schirmer test; (5) antibodies to extractable nuclear antigens (ENA) measured by counter-immunoelectrophoresis using human spleen and calf thymus abstracts as antigens; (6) ESR by Seditainer ESR System; (7) serum creatine kinase (CK); (8) plasma C3 degradation products (C3d) by double-decker immunodiffusion, and (9) abnormal muscle histology as defined below.

MUSCLE BIOPSY

Samples of vastus lateralis from the lateral mid-thigh were obtained under local anaesthesia using a 5 mm diameter Bergstrom percutaneous needle. They were wrapped in a damp saline gauze, transported rapidly to the laboratory and snap frozen for histology. Transverse sections were cut on a cryostat and for each biopsy, five levels stained with haematoxylin and eosin were examined together with histochemical stains for ATPase and NADH Tr. Muscle fibre atrophy was determined qualitatively on ATPase stained preparations (fig 1). Vessel wall thickening was defined as arterioles and capillaries subjectively judged to have abnormally thick and prominent walls, replaced by eosinophilic hyaline material and affecting the majority of vessels in the sample (fig 2). Lymphocytic vasculitis was defined as lymphoid infiltration of the vessel wall in association with endothelial cell swelling with additional perivascular cuffing by lymphoid cells; affecting small vessels (arterioles and small arteries, or small veins) (fig 3). Myositis was defined according to the criteria of Bohan and Peter.⁹

STATISTICAL TESTS

Mann Whitney U test and χ^2 test were used for continuous and discrete variables, respectively, to test differences between groups. All calculations were performed using the programme SPSS for the Macintosh computer version 4.01 (1991). $P < 0.05$ was taken to be significant.

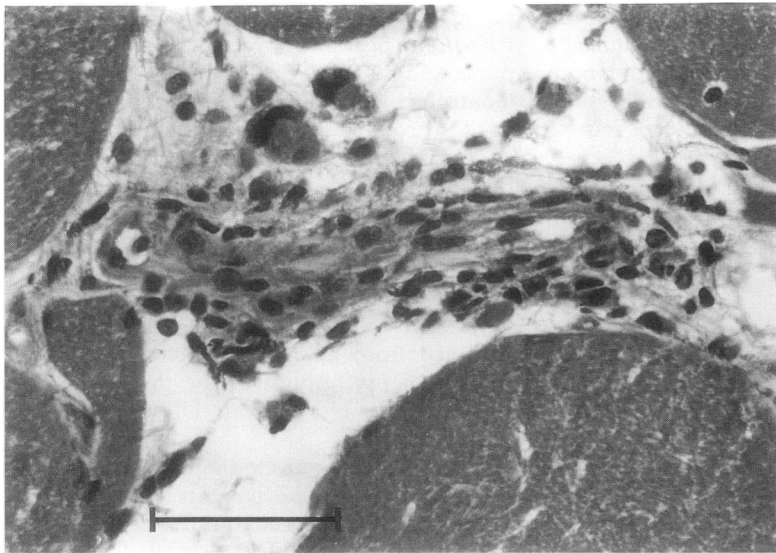


Figure 3 Lymphocytic vasculitis showing infiltration of vessel wall with lymphoid cells and cells extending into adjacent interstitium. Bar = 50 μ m.

Table 1 Selected demographic features and muscle biopsy findings in the SLE patient group and control group

Features	SLE patient group n = 55	Control group n = 26
Age, mean (SD) years	46.8 (14.2)	38.2 (10.1)
% Female	93%	62%
Disease duration, mean (SD) years	6.8 (9.4)	3.5 (4.6)
Other medical conditions	1 thyroid dysfunction	1 diabetes mellitus
Drug history:		
Steroids	9	3
Cytotoxics	4	0
Steroids and cytotoxics	7	0
Untreated/Hydroxychloroquine	35	23
Muscle findings: ¹		
Abnormal (%)	48 (87%)**	15 (58%)
Myositis	7	0
Lymphocytic vasculitis	14**	0
Vessel wall thickening	30	11
Type II fibre atrophy	34*	9

¹ χ^2 test, *P<0.05 **P<0.005. SD = standard deviation.

Results

Demographic features and muscle biopsy findings in the patients with SLE and the control groups are listed in table 1. The control group was generally younger and had a higher male to female ratio than the SLE patient group. One patient in each group had a coincidental endocrine abnormality known to be associated with skeletal muscle changes.

Twenty of the 55 patients with SLE were on immunosuppressive therapy, 16 of whom were taking prednisolone at the time of muscle biopsy. In contrast, three of the controls were taking low dose daily prednisolone (<15 mg), started by their general practitioners for musculoskeletal symptoms.

Abnormal muscle biopsies were significantly more frequent in the SLE patient group compared with the control group (P<0.005). Type II fibre atrophy was significantly increased in patients with SLE (P = 0.02) but there was no significant difference in the incidence of vessel wall thickening between the two groups (P = 0.3). None of the controls had lymphocytic vasculitis or myositis. The difference in incidence of lymphocytic vasculitis between the SLE and the control groups was significant at P<0.005. However, due to the small number of cases, the difference in incidence of myositis between the two groups only reached P = 0.09. None of our 55 patients with SLE showed evidence of vacuolar myopathy or neurogenic muscle damage.

Comparisons of type II fibre atrophy, lymphocytic vasculitis and myositis with clinical and laboratory parameters in patients with SLE are presented in table 2. Similar analyses for vessel wall thickening were not included as this particular finding failed to distinguish patients with SLE from controls. All subgroups were comparable for age and disease duration. A significantly higher incidence of arthritis was associated with lymphocytic vasculitis in the muscle specimens (P = 0.01). The lymphocytic vasculitis group had significantly higher ESR values when compared with patients without lymphocytic vasculitis (P = 0.007) with median 40 mm/hour, range 12–97 and median 21, range 2–125 respectively. Lymphocytic vasculitis in the muscle specimens appear to be associated with an increased incidence of positive Schirmer tests, antibodies to Ro and/or antibodies to La. Muscle specimens of two patients with SLE showed perivascular lymphocytic infiltration only (in the absence of wall inflammation) and were not included in the lymphocytic vasculitis group. All but one patient with myositis had elevated CK values and the median CK value for the myositis

Table 2 Correlation of muscle biopsy abnormalities with clinical and laboratory parameters in the SLE patient group

Parameters	Type II fibre atrophy		Lymphocytic vasculitis		Myositis	
	+ve (n = 34)	-ve (n = 21)	+ve (n = 14)	-ve (n = 41)	+ve (n = 7)	-ve (n = 48)
Age, mean (SD) years	48.5 (14.3)	44.1 (13.9)	45.8 (16)	47.1 (13.7)	47.6 (13.6)	46.7 (14.4)
Disease duration, mean (SD) years	7.8 (9.3)	5.3 (9.7)	8.6 (11.4)	6.2 (8.7)	5.7 (10)	7 (9.4)
% on steroids	29.4%	28.5%	35.8%	26.8%	42.9%	27.1%
Clinical symptoms (%):						
Arthralgia	41.2	57.1	57.1	43.9	28.6	50
Arthritis	29.4	14.3	50*	14.6	28.6	22.9
Myalgia	61.8	38.1	35.7	58.5	57.1	52.1
Proximal weakness	14.7	14.3	21.4	12.2	42.9	10.4
Vasculitis rashes	17.6	19	21.4	17.1	14.3	18.8
Positive Schirmer test (%)	5.9	9.5	14.3	4.9	0	8.3
ENA antibodies (% + ve):						
Ro	25	28.6	50	17.9	14.3	28.3
La	0	23.8	21.4	5.1	0	10.9
RNP	31.3	28.6	28.6	30.8	71.4	23.9
Laboratory parameters:						
ESR (mm/hour)	22 (2–125)	30 (2–89)	40* (12–97)	21 (2–125)	31 (4–77)	22 (2–125)
C3d (units/mL)	12 (6–31)	11 (7–18)	13 (8–24)	11 (6–31)	8 (6–18)	12 (7–31)
CK (IU/L)	73 (1–6696)	59 (1–2008)	64 (1–2008)	70 (1–6696)	902** (71–6696)	57 (1–2008)

*P<0.01 **P<0.005. Median values (range) shown for ESR, C3d, and CK. SD = standard deviation.

group (median 902 IU/L, range 71–6696) was significantly higher compared with patients without myositis (median 57 IU/L, range 1–2008) with $P < 0.005$. There was also an increased incidence of symptoms of proximal weakness and antibodies to RNP in patients with myositis.

None of the other clinical and laboratory parameters were significantly different between patients with and without type II fibre atrophy, lymphocytic vasculitis or myositis. Similarly no significant difference in steroid use was noted between the subgroups.

Discussion

We have documented a significantly higher incidence of abnormalities in muscle biopsy specimens from patients with SLE compared with a group of controls ($P < 0.005$). Oxenhandler *et al*¹⁰ tried to document the incidence and types of muscle abnormalities in patients with SLE but the authors did not include a control group for comparison. Other studies have focused chiefly on specific abnormalities in muscle specimens from patients with SLE.^{11–16}

The absence of lymphocytic vasculitis and/or myositis in the control group suggests that such changes signify pathology related to the presence of SLE. The relationship between lymphocytic vasculitis and clinical and laboratory parameters has not been examined previously. There are conflicting views on the significance of lymphocytic vasculitis in biopsy specimens,^{11 17 18} but our results show that patients with lymphocytic vasculitis had significantly higher ESR values compared with those without lymphocytic vasculitis ($P < 0.01$). The ESR has long been regarded as a marker of disease activity in patients with SLE,¹⁹ and we suggest that lymphocytic vasculitis in biopsy specimens of quadriceps muscle might be associated with more active disease in these patients. A striking finding of this study was the association between the presence of lymphocytic vasculitis in muscle biopsies and arthritis ($P < 0.005$). This observation, coupled with previous reports of similar vascular lesions in the synovium of patients with SLE²⁰ and rheumatoid arthritis,²¹ supports the viewpoint that these lymphocytic vasculitis lesions in muscle are pertinent to the immunopathology of SLE.

Furthermore, a notable relationship between lymphocytic vasculitis and a higher, though not significant, incidence of positive Schirmer tests, anti-Ro antibodies and/or anti-La antibodies was observed. The relevance of this is not apparent but lymphocytic vasculitis in quadriceps muscle biopsy specimens could be a feature of patients with SLE with associated secondary Sjögren's syndrome and the increased incidence of anti-Ro and/or anti-La antibodies in these patients supports this hypothesis.²²

Seven of 55 SLE patients (13%) had an inflammatory myositis and this finding on muscle biopsy correlated well with the higher incidence of proximal weakness and the significantly elevated serum CK levels in these

patients ($P < 0.005$). This suggests that serum CK is a good guide to underlying myositis in patients with SLE. An increased incidence of anti-RNP antibodies was also found in the myositis group and this is in keeping with accepted associations of these antibodies.^{23 24} Anti-RNP antibodies occur in up to 40% of patients with SLE and are found in nearly all patients with mixed connective tissue disease (MCTD) and are also associated with myositis, oesophageal hypomotility, Raynauds phenomenon and lack of nephritis. However, because there is considerable overlap between SLE and MCTD clinically, we did not analyse our data on this basis.²⁵

In contrast to lymphocytic vasculitis and myositis, type II fibre atrophy and vessel wall thickening were found in both SLE and control groups. Type II fibre atrophy was the most common abnormal finding in both the SLE and the control groups but the SLE patient group had significantly higher incidence of type II fibre atrophy compared with the controls. However, this result must be interpreted with care in view of the age and sex differences between the two groups as, apart from connective tissue diseases, type II fibre atrophy can be associated with disuse, steroid therapy and is influenced by age and sex.^{26–28} In this study, steroid use did not appear to differ between those patients with and those without type II fibre atrophy. The higher than expected incidence of type II fibre atrophy in the control group is possibly a result of physical inactivity of their conditions.

We found no significant difference in the incidence of vessel wall thickening between the SLE and the control groups. Similarly, Pallis *et al* working in our laboratory, using a new computer-aided quantitative assessment of capillary basement membrane thickness on electron photomicrographs, showed no significant difference in capillary basement membrane thickness between a further group of 22 patients with SLE and 11 controls (unpublished data). A variety of chemical, radiological and bacterial insults can result in vessel wall thickening.²⁹ Norton has characterised vessel wall thickening in SLE, dermatomyositis, diabetes mellitus and scleroderma and noted that the changes were more frequent than in normal subjects.³⁰ The disparity between Norton's observations and ours could be attributed to the entirely subjective measure of vessel wall thickening used. Consequently vessel wall thickening was not included in the further analyses.

In summary, although we have demonstrated a significantly higher incidence of abnormal findings in needle quadriceps muscle biopsy specimens in patients with SLE, lymphocytic vasculitis alone appears to characterise a subset of patients with positive Schirmer tests, anti-Ro antibodies, anti-La antibodies and/or arthritis. Lymphocytic vasculitis may prove to be a valuable marker of disease activity in patients with SLE. Raised serum CK corresponded with underlying myositis in SLE patients. In contrast, both type II fibre atrophy and vessel wall thickening appear to be non-specific

findings. We conclude that histopathological abnormalities in needle quadriceps muscle biopsies are further valuable parameters in the assessment of patients with SLE.

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