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Polymyositis with cytochrome oxidase negative muscle fibres

Early quadriceps weakness and poor response to immunosuppressive therapy

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Summary

We evaluated 10 patients with histologically typical polymyositis except for an excess of muscle fibres with absent cytochrome oxidase (COX) staining. No biopsies had vacuoles or congophilic material in muscle fibres. All patients presented with a history of slowly progressive weakness. The average age of onset was 9 years older than a group of polymyositis patients with normal COX staining of muscle fibres. Selective weakness of knee extension was a prominent and disabling feature in most patients. Serum creatine kinase was usually mildly elevated (363±115 U/l) but at levels lower than those in other patients with polymyositis. Mitochondrial DNA analysis showed multiple deletions in 90% of muscles from patients with excessive numbers of COX-negative muscle fibres, a prevalence significantly greater than the 22% figure for controls (P = 0.005). As a group, the patients responded poorly to immunosuppressive therapy. We conclude that patients with polymyositis and an excess of COX-negative muscle fibres, but no inclusion bodies, have common features including selective quadriceps weakness, mitochondrial pathology by histochemical and DNA analysis and a poor response to immunosuppressive therapy. Some of these features are shared with inclusion body myositis (IBM) and this entity cannot be entirely excluded as vacuoles may not be present in all muscle tissue in IBM patients. Evaluation of the COX activity in muscle fibres of patients with inflammatory myopathies provides useful prognostic information regarding the likelihood of improved strength after immunosuppressive treatment.

Keywords: muscle; polymyositis; mitochondria; cytochrome oxidase; autoimmune

Abbreviations: CK = creatine kinase; COX = cytochrome oxidase; H&E = haematoxylin and eosin; IBM = inclusion body myositis; mtDNA = mitochondrial DNA; PCR = polymerase chain reaction; SDH = succinate dehydrogenase

Introduction

The inflammatory myopathies are defined by a set of characteristics including muscle weakness, elevated levels of creatine kinase (CK) in serum, myopathic potentials with irritability on electrodiagnostic testing, and myopathic changes with inflammation on pathological examination of muscle (Plotz *et al*., 1989; Dalakas, 1994). Muscle pathology also provides a basis for subdividing the inflammatory myopathies (Carpenter and Karpati, 1992). Perifascicular atrophy strongly supports a diagnosis of dermatomyositis. Vacuoles, rimmed by granular basophilic material, in muscle fibres suggest inclusion body myositis (IBM). In the absence of these two pathological findings, an immune-mediated inflammatory myopathy is usually categorized as polymyositis.

Several studies have suggested that mitochondrial changes are more common in IBM than in polymyositis. Mitochondrial DNA (mtDNA) deletions have been found in at least half of patient populations with IBM (Oldfors *et al*., 1993, 1995). The mitochondrial abnormalities in IBM are manifest by an excess of muscle fibres with deficient cytochrome oxidase (COX) activity and ragged red fibres with excessive staining for succinate dehydrogenase (SDH) (Oldfors *et al*., 1993). Mitochondrial abnormalities have been described in polymyositis without inclusion bodies (Watkins and Cullen,

1987; Campos *et al*., 1995), but are generally thought to be unusual (Rifai *et al*., 1995).

The routine evaluation of muscle biopsies at Washington University, St Louis, includes staining for COX activity. Over a 5-year period, we noted that an unexpectedly high number of biopsies (10, i.e. 21%) from patients with diagnoses of polymyositis frequently showed muscle fibres with deficient COX activity (>3% of the total number). These biopsies did not show vacuoles on serial haematoxylin and eosin (H&E) stained sections, nor did they show congophilic material, with or without vacuoles, on Congo-red stained sections under a polarized filter. We report the clinical features and mtDNA abnormalities in these 10 patients.

Method

Muscle biopsies

All muscle specimens were removed by an open biopsy procedure and evaluated by a neuromuscular specialist (A.P.) at Washington University, St Louis. Biopsy features necessary to support a diagnosis of polymyositis included inflammatory cell infiltrates, especially in endomysial regions, and myopathic changes, including variation in muscle fibre size, and some evidence of disease activity, such as necrosis with phagocytosis and regenerating muscle fibres. Increased amounts of endomysial connective tissue were present in biopsies from patients with longer histories of weakness. For this study, the presence of even one muscle fibre containing a rimmed vacuole in the biopsy excluded patients from categorization as typical polymyositis. The possibility of sampling error was minimized by the large size of the biopsies, usually containing at least 1000 muscle fibres, and by the examination of multiple sections from each muscle. Polymyositis biopsies were subdivided into groups with normal or excess numbers (>3%) of muscle fibres with deficient COX activity (12 and 10 patients, respectively).

Muscle biopsies were quickly frozen in isopentane cooled with liquid nitrogen. Cryostat-cut cross sections of muscle were stained with the following: H&E; Gomori's modified trichrome; NADH-tetrazolium reductase; ATPase after incubation at pH 9.4, 4.6 or 4.3; Verhoff-van Giesen; periodicacid–Schiff; COX; SDH; acid phosphatase; alkaline phosphatase; non-specific esterase; Congo red; Sudan black; amylophosphorylase (modified Takeuchi method). To determine the percentage of muscle fibres that were COX-negative, a randomly selected region of 300 muscle fibres cut in cross-section was evaluated at ×40 magnification. The number of fibres with negative COX staining was divided by the total number of muscle fibres.

Patients

The diagnosis of polymyositis, with or without COX-deficient muscle fibres, was made by standard criteria prior to, and independently of, mtDNA analysis (Carpenter and Karpati, 1992). The 10 patients with polymyositis and COX-negative muscle fibres shared clinical and laboratory features that are usually part of inflammatory myopathy syndromes, including proximal weakness, myopathic electromyograms with 'irritative' features such as fibrillations and positive sharp waves, and elevated levels of serum CK. Nine of these 10 patients were examined at the Neuromuscular Clinic at Washington University School of Medicine, St Louis. Detailed clinical and electrodiagnostic records were available on all 10 COX-negative polymyositis patients as well as on six of the 12 polymyositis patients without COX-deficient fibres. Controls included dermatomyositis (10 patients), an age-matched group of 13 patients with no primary muscle disease (nine with denervation on muscle biopsy and four normals), and two patients with vacuolar myopathies.

Strength was measured bilaterally in nine muscle groups (arm abduction, elbow flexion, elbow extension, wrist extension, hip flexion, knee extension, knee flexion, ankle dorsiflexion and hand grip) using a hand-held dynamometer, as previously described (Pestronk *et al*., 1994). Results, in pounds, for each muscle were divided by the expected strength for an adult of the same sex in that muscle (from standards established in the Washington University neuromuscular clinic) and multiplied by 100 to obtain a percentage of normal. An overall percentage of normal was determined by deriving an average of the data from individual muscles. A change in strength of ⩾12 units is significant.

Mitochondrial DNA methods

Total DNA was extracted from skeletal muscle by standard proteinase K and SDS (sodium dodecyl sulphate) methods. The presence of mtDNA deletions was analysed by the widely interspaced primer polymerase chain reaction (PCR) technique (Johns and Hurko, 1989; Johns *et al*., 1989). Briefly, the entire mitochondrial genome is screened for deletions using a set of oligonucleotide primer pairs that are spaced too widely apart for amplification of normal mtDNA. However, in the presence of a deletion that does not include either primer, significant amplification of deleted mtDNA will occur. Using standard PCR conditions, the resultant widely interspaced PCR products are visualized in agarose gels.

Illustrative case history

A 53-year-old female (Patient 3) presented for re-evaluation of polymyositis. An elevated serum CK of 709 was discovered during an evaluation for anaemia 15 years previously. At that time, she had no complaints related to disorders of muscle. Six years prior to presentation, she began to notice difficulty with climbing stairs. A diagnosis of polymyositis was based upon a myopathic EMG and right thigh muscle biopsy with myopathic changes and cellular infiltrates. Prednisone treatment for several years produced no benefit. Her weakness progressed, necessitating a cane for ambulation

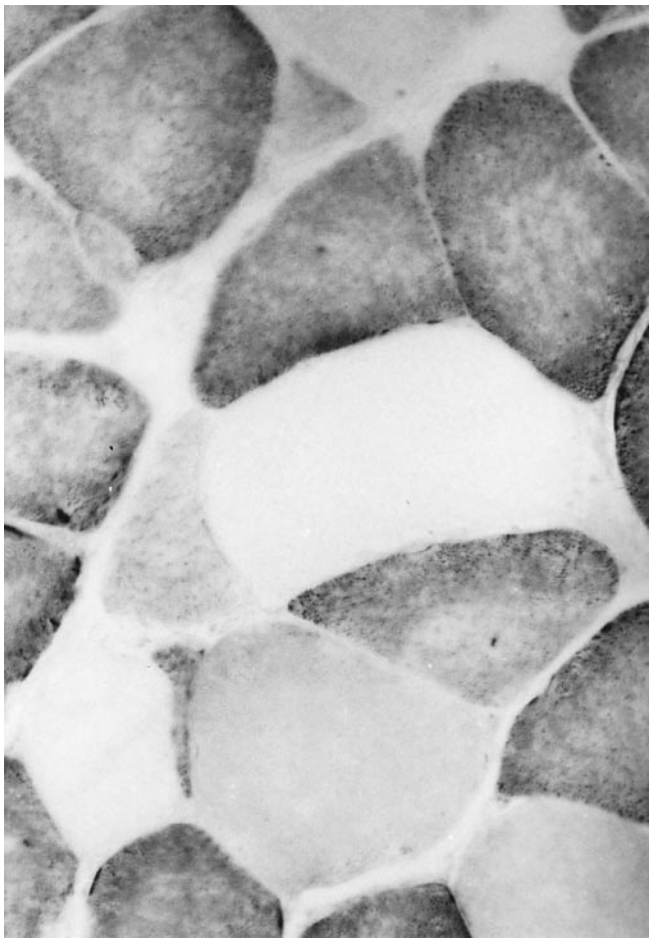

Fig. 1 Patient 4, left deltoid biopsy. Two muscle fibres (a large fibre in the centre of the field and a smaller fibre in the lower left hand corner) with absent or markedly reduced COX activity. Other type I and type II muscle fibres have normal degrees of staining. COX stain; magnification ×400.

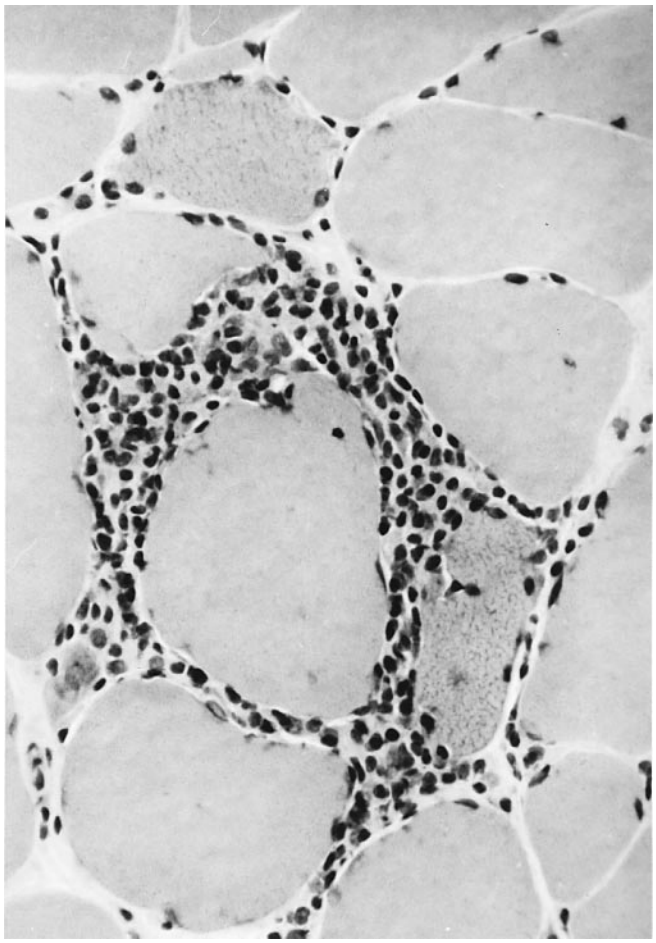

Fig. 2 Endomysial inflammatory cell infiltrate: note focal invasion of two muscle fibres. Two smaller, darker fibres (basophilic) are probably regenerating fibres. H&E stain; magnification ×400.

and causing several serious falls. Side effects of long-term prednisone treatment included easy bruising and bone fractures. Physical examination revealed an obese female with a resting blood pressure of 160/100. General medical examination was unremarkable. Cranial nerve examination showed mild bilateral orbicularis oculi weakness, but was otherwise unremarkable. Diffuse limb weakness was greater proximally (Medical Research Grade, 3+/5) than distally (4/5). Deep tendon reflexes were absent at the ankles, but otherwise unremarkable. Pain and vibratory sensation were mildly reduced distally in the feet. Co-ordination was intact. Gait was waddling and unsteady. A repeat EMG showed myopathic units with irritability. The muscle biopsy showed endomysial inflammatory infiltrates, variation in muscle fibre size, and a mild increase in endomysial connective tissue. COX activity was absent in an excessive number of muscle fibres (5.6%) scattered throughout the biopsy. Addition of azathioprine at a dose of 250 mg/day produced no notable change in strength.

Results

Pathology

In the 10 patients with polymyositis and COX abnormalities, 4.2–27.4% of the total muscle fibres were COX-negative (Fig. 1). In each of the normal subjects and control patients (with diseases other than vacuolar myopathies), including the other 12 with polymyositis, COX-negative muscle fibres accounted for $<0.5\%$ of the total. In one patient (Patient 7) a muscle biopsy that had been performed 10 years previously showed more inflammation, but many fewer COX-negative muscle fibres, than the later biopsy. Fibres with excessive SDH staining, consistent with mitochondrial proliferation, were found in most of the biopsies from patients in the COX-negative polymyositis group. Most muscle fibres with excessive SDH staining were COX-negative. However, many COX-negative muscle fibres, 50–80%, had no clear excess of SDH staining. H&E stains in all 22 patients with polymyositis showed typical endomysial cellular infiltrates and myopathic changes (Fig. 2). Inflammatory infiltrates present in biopsies

Table 1 *mtDNA deletions in different clinical groups*

Clinical group	Number of patients (total)	Patients with mtDNA deletions
Polymyositis		
with COX-negative fibres	10	9 (90%)
with normal COX staining	12	1 (8%)
Dermatomyositis	10	2 (20%)
Age-matched controls		
All controls	13	3 (23%)
Denervated controls	9	3 (33%)
Normal controls	4	0 (0%)
Vacuolar myopathy	2	2 (100%)

from patients with COX-negative polymyositis were indistinguishable histochemically from other polymyositis patients. COX-negative muscle fibres were usually not associated with inflammatory infiltrates or necrotic changes.

Mitochondrial DNA testing

Of the 10 polymyositis biopsies with an excess of COX-negative muscle fibres, multiple mtDNA deletions were found in nine (90%) (Table 1). The biopsy without mtDNA deletions, from Patient 8, had a similar morphological appearance to those with deletions. The biopsy performed 10 years earlier on Patient 7, which had few COX-negative muscle fibres, did not have multiple mtDNA deletions. The frequencies of multiple mtDNA deletions in the control groups (Table 1) were 8% for COX-positive polymyositis, 20% for dermatomyositis and 23% for age-matched controls with no primary muscle pathology. The two patients with vacuolar myopathies, presumably IBM, had multiple mtDNA deletions.

The multiple mtDNA deletions in the polymyositis patients ranged from 2 to 10 kb in length. They were predominantly clustered near an upstream breakpoint at nucleotide position 16 058–16 070 in the non-coding displacement-loop. The majority of the deletions were bounded by small, imperfect direct repeats, but some deletion junctions contained larger, perfect direct repeats or had no direct repeat. In four patients mtDNA was also assayed by Southern blot methodology which confirmed the presence of deletions (S. Dimauro, unpublished observations).

Clinical correlations (Table 2)

The 10 patients with polymyositis and COX-negative muscle fibres had an average age at onset of disease of 61 ± 3 years. This is significantly older ($P = 0.02$) than the average age of the other 38 adults who had a muscle biopsy and polymyositis diagnosed at our centre during the same time period (52 ± 3 years). Overall, progression of weakness was slow in the COX-negative polymyositis group. Serial quantitative strength measurements were made in three patients. They declined by an average of 4% of predicted normal strength per year. Over a period of 4 years, overall strength declined from 59% of normal to 53% in Patient 4, 47% to 28% in Patient 7 and 60% to 41% in Patient 10. The fastest decline, lowest level of residual strength and the region of weakness resulting in the most disability was in knee extension. Knee extension strength declined by an average of 8% per year, twice as fast as overall strength. Over 4 years, knee extension strength declined from 71% to 43% in Patient 4, 32% to 13% in Patient 7 and 55% to 4% in Patient 10.

Disease duration prior to biopsy in the patients with polymyositis and COX-negative muscle fibres ranged from 6 to 120 months. The control polymyositis patients without COX-negative fibres had disease durations ranging from 3 to 72 months, not significantly different from the COX-negative polymyositis patients.

Serum CK measurements were only moderately elevated in the COX-negative polymyositis group, averaging 363 ± 115 U/l, with the highest value being 1200 U/l. In polymyositis patients with normal COX staining of muscle fibres, >71% of patients had serum CK values >1200 U/l ($P = 0.003$).

A final characteristic of the COX-negative polymyositis patients was a poor response to prednisone treatment. None of the eight patients who were treated with prednisone had significant improvement on quantitative muscle testing, or any history of functionally useful benefit. At the time of their last evaluation most patients had either discontinued prednisone entirely, or been tapered to low dosages as new immunosuppressive medications were added. This is in contrast to the polymyositis-control patients of whom five out of six had been treated with corticosteroids, and four of those five showed significant improvement in strength. The patient without improvement had the longest disease course (6 years). No improvement could be documented after treatment of COX-negative polymyositis patients with other immunosuppressive medications, including azathioprine (three patients), methotrexate (two patients), cyclosporine (two patients) and intravenous immunoglobulin (one patient).

Discussion

We studied 10 patients with inflammatory myopathies who had a large excess of muscle fibres with negative staining for COX. The percentage of the COX-negative muscle fibres was ⩾4% in each of the 10 biopsies. This figure is far above the range for normal for age-matched controls (< 0.5%) from previously published data and from our laboratory (Muller-Hocker, 1990; Rifai *et al*., 1995). The range of frequencies of COX-negative muscle fibres in these 10 patients (4.2–27.4%) is also considerably higher than those reported in a series of patients with late-onset mitochondrial myopathy (Johnston *et al*., 1995). Only one of their nine patients had >3% of muscle fibres with COX-negative staining. Ragged red fibres and COX-negative muscle fibres have been described previously in inflammatory myopathies, but have not been quantified or correlated with clinical

Table 2 *Clinical data: polymyositis patients with COX-negative fibres*

Patient	mtDNA deletion	Sex	Age at biopsy (years)	Disease duration (years)	Weakness distribution				Initial serum CK	%COX negative fibres
					P	D	S	F		
1	+	F	62	2	+	+	+	−	415	6.2
2	+	F	71	3	+	−	+	−	54	4.2
3	+	F	53	6	+	−	−	+	216	5.6
4	+	M	63	1	+	−	+	+	874	6.1
5	+	F	81	10	+	+	+	+	418	10.8
6	+	F	80	11.5	+	−	+	−	358	4.5
7	+	M	67	1	+	−	+	−	965	6.4
8	−	M	70	4	+	+	+	+	323	6.3
9	+	M	47	4	+	−	+	+	1200	27.4
10	+	F	63	0.5	+	+	−	−	463	4.3

CK = serum creatine kinase in U/l. Weakness distribution: P = proximal, D = distal, S = symmetric, F = facial. % COX-negative fibres = percentage of 300 randomly selected fibres with no staining for cytochrome oxidase.

outcome (Mikol and Engel, 1994; Rifai *et al*., 1995). In one series of polymyositis and dermatomyositis patients, ragged red fibres were quantified; seven out of eight of the older patients (age $\geqslant$39 years) had $<$1% ragged red fibres (defined by excessive SDH staining) and 'a small proportion' of COX-negative fibres which were not ragged red fibres (Rifai *et al*., 1995). One patient had 4.85% ragged red fibres; none of the younger patients (age $<$39 years) had $>$0.2% ragged red fibres.

The presence of an excess of COX-negative muscle fibres in our myositis patients correlated with the presence of mtDNA deletions in muscle. The mtDNA deletions occurred in 90% of patients with polymyositis and COX-negative muscle fibres. The two older patients with vacuolar myopathies, with or without inflammation, formed another small group that had mtDNA deletions (two out of two; 100%). These two patients also had a high frequency of COX-negative muscle fibres. It has been reported that mtDNA deletions occur frequently in patients with IBM, a disorder that has vacuolar changes in muscle fibres identical to those in these two patients (Oldfors *et al*., 1993, 1995). The other patient groups, none of which had an excess of COX-negative muscle fibres, had a much lower prevalence of mtDNA deletions ($P = 0.005$), with 8% in polymyositis and normal COX staining, and 23% in age-matched controls without primary muscle disease.

Multiple mtDNA deletions in a tissue are generally found in autosomally transmitted and sporadic mitochondrial diseases (Zeviani *et al*., 1989; Cormier *et al*., 1991; Ohno *et al*., 1991; Suomalainen *et al*., 1992; Hirano *et al*., 1994). The multiple mtDNA deletions in our polymyositis patients with COX-negative fibres were probably an acquired trait. The sequential biopsies in Patient 6, with the second but not the first showing mtDNA deletions, support this idea. The initial biopsy showed myopathic changes and endomysial inflammation, a combination of changes that was interpreted as being typical of polymyositis. COX-negative muscle fibres were sparse, similar to the frequency seen in our control groups. The biopsy conducted 11 years later, as part of a work-up for a corticosteroid-resistant myopathy, revealed more chronic appearing myopathic changes with increased endomysial fibrosis. There was less inflammation but numerous COX-negative muscle fibres were present.

Mutations producing multiple mtDNA deletions are known to develop in skeletal muscle with normal ageing (Harding, 1992; Simonetti *et al*., 1992; Wallace, 1992; Lee *et al*., 1994). In IBM muscle, multiple mtDNA deletions are thought to arise clonally and to be propagated in individual muscle fibres (Oldfors *et al*., 1995). Postulates regarding stimuli for the development of deletions have included free-radical generation, and abnormal accuracy, or rates, of mtDNA replication (Corral-Debrinski *et al*., 1992; Soong *et al*., 1992). The mitochondrial abnormalities in our myositis patients could either be a primary event that then stimulates myopathy and inflammatory infiltration, or be secondary to, and stimulated by, the inflammation, or muscle fibre degeneration and regeneration. Mitochondria with DNA deletions are felt to have an advantage during replication leading to increased numbers relative to mitochondria with wild-type DNA (Yoneda *et al*., 1992).

Whatever the mechanisms of development of the mitochondrial abnormalities, our 10 patients with inflammatory myopathies and COX-negative muscle fibres had clinical features suggestive of IBM rather than polymyositis (Yunis and Samaha, 1971; Danon *et al*., 1982). Their clinical characteristics included presentation later in life (average age, 61 years) than other patients with polymyositis, slow progression of weakness, selective weakness of knee extension that became more apparent as the disease evolved, and little or no improvement during immunosuppressive treatment. A few patients had weakness of the tibialis anterior muscle. However, our patients generally did not have much early involvement of ankle dorsiflexion or wrist flexion, two muscle groups often identified as weak in IBM. On laboratory testing, most of the COX-negative patients had only mildly elevated serum CK. The average CK in this group was 363 U/l serum, $<$10% of the average

value of 3846 U/l serum in our polymyositis patients with normal staining of COX in muscle fibres.

According to a recently published set of clinical criteria, our patients would have been classified as 'possible' IBM (Griggs *et al*., 1995). The clinical similarities between patients with polymyositis with COX-negative muscle fibres and IBM suggest that these may be variants of the same disease process. Electron microscopy has been used to look for paired-helical filaments as pathological diagnostic criteria in IBM (Griggs *et al*., 1995). More recently, immunostaining with anti-neurofilament antibody has been used to detect paired-helical filaments, but in the absence of vacuoles this technique is not often positive (Askanas *et al*., 1996). Accurate definition of the degree of pathogenetic overlap between our patient group and IBM probably awaits better definition of the underlying causes of the mitochondrial abnormalities.

The poor response to immunosuppressive treatment suggests that it may be useful to identify patients with COX-negative muscle fibres as a separate subgroup of polymyositis, whether or not inclusion bodies are found. The risk-to-benefit ratio for immunosuppressants is much higher in patients with COX-negative fibres than in the group of polymyositis patients as a whole. This prognostic issue merits consideration before aggressive trials of immunosuppressive medications are undertaken. The activity of COX in muscle fibres is easily evaluated by histochemical staining, and should probably be studied whenever polymyositis is suspected on clinical or pathological grounds. In several of our patients, corticosteroid therapy had been continued for many years despite a lack of response to the treatment, with slowly progressive loss of strength and functionally significant side effects. COX staining of muscle, in concert with mtDNA analysis, may provide a rationale for discontinuing immunosuppressive treatment when reevaluating patients with otherwise typical polymyositis who are unresponsive to therapy.

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