Does previous corticosteroid treatment affect the inflammatory infiltrate found in polymyositis muscle biopsies?

M.M. Pinhata[1], J.J. Nascimento[1], S.K.N. Marie[2], S.K. Shinjo[1]

[1]Division of Rheumatology, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; [2]Laboratory of Molecular and Cellular Biology, Department of Neurology, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

Abstract

Objective

The aim of the study was to evaluate the effect of the prior use of corticosteroids (CS) on the presence of inflammatory infiltrates (InI) in muscle biopsies of polymyositis (PM).

Methods

We retrospectively evaluated 60 muscle biopsy samples that had been obtained at the time of the diagnosis of PM. The patients were divided into three groups according to the degree of the InI present in the muscle biopsies: (a) minimal InI present only in an interstitial area of the muscle biopsy (endomysium, perimysium) or in a perivascular area; (B) moderate InI in one or two areas of the interstitium or of the perivascular area; and (C) moderate InI throughout the interstitium or intense inflammation in at least one area of the interstitium or of the perivascular area.

Results

The three groups were comparable regarding the demographic, clinical and laboratory features (p>0.05). Approximately half of the patients in each group were using CS at the time of the muscle biopsy. The median (interquartile) duration of CS use [4 (0-38), 4 (0–60) and 5 (0–60) days: groups A, B and C, respectively] and the median cumulative CS dose used [70 (0–1200), 300 (0–1470) and 300 (0–1800)mg] were similar between the groups (p>0.05).

Conclusion

Previous CS use did not influence the presence or the degree of InI found in muscle biopsies in PM with clinical and laboratory disease activity. Our study showed that muscle biopsies should be performed this population, even in individuals who have already been taking CSs.

Key words

corticosteroid, inflammatory myopathies, muscle biopsies, polymyositis

Mayara M. Pinhata, MD
Juliana J. Nascimento, Biol
Suely K.N. Marie, PhD
Samuel K. Shinjo, PhD

Please address correspondence and reprint requests to:
Samuel Katsuyuki Shinjo,
Av. Dr. Arnaldo, 455,
3º andar, sala 3150,
CEP 01246-903, São Paulo, Brazil.
E-mail: samuel.shinjo@gmail.com

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Introduction

Polymyositis (PM) is a systemic inflammatory autoimmune myopathy characterised by the subacute onset of symmetric proximal limb muscle weakness leading to a functional disability (1-5). In addition to this clinical manifestation, i) elevated serum levels of skeletal muscle enzymes, ii) electromyographic evidence of a classic pattern of muscular impairment and iii) a characteristic muscle biopsy could be used to define PM. Within this context, the biopsy could show inflammatory cells surrounding multiple myofibres and an absence of rimmed vacuoles and a combination of myofibre degeneration, regeneration and / or necrosis (1, 2).

The treatment of PM is typically based on corticosteroids (CS) and/or immunosuppressants (including methotrexate, azathioprine and cyclosporine) (6). The early introduction of such medications, especially CS, might enable faster and more effective control of the disease activity of PM, leading to a change in the prognosis and to morbidity and mortality minimisation.

Although it has not been scientifically proven, the early introduction of CS might immediately interfere in the inflammatory process in the muscle tissues of patients with PM. Physicians have been postponing the introduction of drug therapy until after the muscle biopsy is performed to avoid possibly obscuring the histologic diagnosis. These biopsies have been avoided in the patients who have already been using CS because the CS might obscure the more suggestive signs of inflammatory myopathies, and the biopsy could be an unnecessary surgical procedure.

Some studies have provided indirect evidence that inflammatory cell infiltrations in muscle biopsies persist despite longer courses of CS treatment (7, 8). Another study, however, showed that the presence of inflammatory cells decreases after pulse therapy with intravenous methylprednisolone (9).

To further define the effect of previous CS and/or immunosuppressants treatments on inflammatory infiltrations in muscle biopsy specimens, this study systematically reviewed the treatment regimens and histological muscle biopsies of the patients with PM. This study also analysed whether there is a correlation between the inflammatory infiltrations in muscle biopsies and the severity of PM disease activity.

Patients and methods

This retrospective study initially included 75 adult patients admitted to our tertiary center from 2002 to 2013 to investigate the clinical manifestations of progressive symmetrical muscle weakness of all four limbs associated with high serum levels of skeletal muscle enzymes. None of our patients had neoplasia, a family history of muscle disease, overlapping systemic autoimmune diseases or PM-like diseases (*i.e.* muscular dystrophies, inclusion body myositis and immune-mediated necrotising myopathies). Moreover, none of patients had used statins or fibrates.

All of the patients had electromyography results suggesting a pure inflammatory myopathy. Moreover, 60 of out 75 patients had muscle biopsies with signs of myopathy (fibres with regeneration, degeneration, necrosis, connective tissue alterations and different degrees of the inflammatory cell infiltrations) and without evidence of rimmed vacuoles in the myofibres. Thus, we analysed these 60 patients who fulfilled all of the Bohan and Peter criteria (1, 2).

The study was approved by the local Research Ethics Committee of our University Hospital, and the data were collected from our parameterised and standardised electronic medical records of these 60 patients. The data included the following information that was relevant to this study:

a) Demographic data: gender, age of onset-PM, ethnicity, time duration between the onset of diagnosis and symptoms of PM;
b) Clinical manifestations: constitutional symptoms, cardiorespiratory, gastrointestinal and musculoskeletal involvement prior to the muscle biopsy. The limb muscle strength was graded according to the Medical Research Council – grade 0: absence of muscle contraction, grade I: slight signs of contractility, grade II: movements of normal amplitude but not against gravity, grade III: nor-

mal range of motion against gravity, grade IV: full mobility against gravity and a degree of resistance and grade V: complete mobility and strong resistance against the action of gravity (10);

c) Treatment: the dose and duration of CS (prednisone or methylprednisolone pulse therapy) treatment and/or immunosuppressants prior to the muscle biopsy;

d) Laboratory data: serum levels of muscle enzymes [creatine kinase (normal range: 24–173 U/L), aldolase (1.0–7.5 U/L), alanine aminotransferase (<41 U/L), aspartate aminotransferase (<37 U/L), lactic dehydrogenase (240–480 U/L)] determined by the automated kinetic method and collected at the time of the muscle biopsy. The antinuclear antibody (ANA) was determined by immunofluorescence using Hep-2 cells. The anti-Jo-1 antibody was determined by immunoblotting.

Muscle biopsies were performed routinely at the time of the PM diagnosis in the biceps brachii or in the vastus lateralis muscle of the thighs, and the samples were subjected to routine standard histological techniques. In the present study, we re-analysed the muscle biopsies stained with haematoxylin and eosin (H&E).

Each muscle biopsy specimen was coded and analysed separately by two investigators (M.M.P. and J.J.N.) who were blinded to the patient data. When a discrepancy was noted, the specimen was reviewed by a third investigator (S.K.S.) to reach a general consensus.

The following parameters were assessed semi-quantitatively as minimal, moderate or intense: fibre features (presence of fibre degeneration, regeneration or necrosis); increased connective tissue (endomysial and/or perimysial); and the degree of inflammatory cell infiltration in the perimysial, endomysial and/or perivascular area. We classified the patients with PM into three groups (A, B and C) according to the presence of inflammatory cell infiltrates in the muscle biopsy specimens:

- Group A: minimal presence of inflammatory cell infiltrates restricted to one interstitial muscle biopsy area (endomysial, perimysial or perivascular);
- Group B: moderate presence of inflammatory cell infiltrates in one or two muscle biopsy interstitial areas;
- Group C: i) moderate presence of inflammatory cell infiltrates in all of the interstitial areas or ii) intense presence of inflammatory cell infiltrates in at least one interstitial muscle biopsy area.

Table I. General features of the patient groups (groups based on the inflammatory grade of the muscle biopsy).

Parameters	Group A (n=10)	Group B (n=23)	Group C (n=27)	*p*-value
Age at disease onset (years)	42.6±11.1	46.1±16.3	40.1±14.0	0.352
Female gender	6 (60.0)	17 (73.9)	18 (66.7)	0.709
White ethnicity	7 (70.0)	21 (91.3)	21 (76.8)	0.217
Time between diagnosis and symptoms (months)	1.5 (0.0-5.3)	6.0 (1.0-12.0)	4.0 (3.0-12.0)	0.104
Constitutional symptoms	5 (50.0)	14 (60.9)	15 (55.6)	0.837
Dysphagia	2 (20.0)	8 (34.8)	8 (29.6)	0.709
Dysphonia	0	1 (4.3)	2 (7.4)	0.696
Articular involvement	4 (40.0)	13 (56.5)	12 (44.4)	0.645
Pulmonary involvement	5 (50.0)	3 (13.0)	3 (11.1)	0.661
Muscle strength				0.052
Upper limbs				
Grade V	2 (20.0)	2 (8.6)	0	0.085
Grade IV	5 (50.0)	17 (73.9)	18 (66.7)	0.306
Grade III	3 (30.0)	3 (13.0)	9 (33.3)	0.383
Grade II	0	1 (4.3)	0	0.441
Lower limbs				
Grade V	0	0	0	1.000
Grade IV	7 (70.0)	23 (100.0)	14 (51.8)	0.001
Grade III	3 (30.0)	3 (13.0)	12 (44.4)	0.054
Grade II	0	1 (4.3)	1 (3.7)	0.807
Antinuclear antibody	8 (80.0)	13 (56.5)	9 (33.3)	0.097
Anti-Jo1 antibody	2 (20.0)	4 (17.4)	2 (7.4)	0.734
Anti-PL-7 antibody	0	1 (47.3)	0	
Anti-SRP antibody	0	1 (4.3)	2 (7.4)	
Anti-Ro-52 antibody	2 (20.0)	4 (17.4)	5 (18.5)	
CPK (U/L)	2965 (1448-8443)	1968 (1103-3506)	3446 (1800-9844)	0.245
Aldolase (U/L)	23.3 (10.6-48.6)	30.7 (18.5-43.0)	45.9 (12.6-115.1)	0.480
ALT (U/L)	87 (46-277)	41 (35-97)	177 (58-223)	0.185
AST (U/L)	77 (42-223)	67 (43-115)	114 (82-243)	0.357
LDH (U/L)	809 (612-1280)	751 (526-1051)	1227 (810-1898)	0.199

Results expressed in the percentage (%), mean ± standard deviation or median (25^{th} - 75^{th} interquartile). ALT: alanine aminotransferase; AST: aspartate aminotransferase; CPK: creatine phosphokinase; LDH; lactic dehydrogenase.

Statistical analysis

The Kolmogorov-Smirnov test was used to evaluate the distribution of each parameter. The demographic and clinical features are expressed as the mean and standard deviation (SD) for continuous variables or as frequencies and percentages for categorical variables. The median (25^{th} – 75^{th} percentile) was calculated for the continuous variables that were not normally distributed. Parameter comparisons among the three groups (A, B and C) were made using ANOVA or Kruskall Wallis test for continuous variables. Values of $p<0.05$ were considered significant. All of the analyses were performed with SPSS 15.0 statistics software (Chicago, USA).

Results

Sixty patients with PM were systematically evaluated and divided into three groups (A, B and C) based on the findings of the degree of inflammatory infiltrates in muscle biopsies (Table I).

Unlike the A and B groups, the, histological findings of group C had a higher frequency of necrotic fibres, hyaline, basophilic and necrotic fibres that were invaded by lymphomononuclear cells. Group C had the greatest area of thick-

ened endomysial and perimysial tissue. The three groups were comparable regarding the age at the time of the muscle biopsy, the distribution of gender and ethnicity, the time interval between the diagnosis of PM (muscle biopsy) and the onset of symptoms ($p>0.05$), as shown in Table I.

The presence of constitutional symptoms early in the disease and the presence of dysphagia, dysphonia, lung and joint involvement, the degree of muscle weakness and the serum levels of muscle enzymes were similar among the three groups ($p>0.05$).

Approximately half of the patients in each group were using CS at the time of the muscle biopsies (50.0%, 50.2% and 63.0%, respectively, in groups A, B and C), as shown in Table II. The time of CS use and the cumulative CS dose were similar among the groups ($p>0.05$).

The maximum duration of CS use by patients in each group were 90, 365 and 270 days, respectively, in groups A, B and C, and the maximum cumulative CS doses were 1.8, 12.8 and 10.8 g.

Three out of 60 patients had also received immunosuppresants prior to muscle biopsies. One from Group A (methotrexate 10 mg/week, 2 months) and two from Group B (methotrexate 20 mg/week, 2 months; methotrexate 20 mg/week, 5 months).

Table II. Prior corticosteroid treatment in the patient groups (groups based on the inflammatory grade of the muscle biopsy).

Prednisolone	Group A (n=10)	Group B (n=23)	Group C (n=27)	*p*-value
Current use	5 (50.0)	12 (52.2)	17 (63.0)	0.668
Time (days)	4 (0-38)	4 (0-60)	5 (0-60)	0.848
Cumulative dose (mg)	70 (0-1200)	300 (0-1470)	300 (0-1800)	0.649

Results expressed in the percentage (%) or the median (25^{th}-75^{th} interquartile).

Discussion

This study showed that the previous use of CS was not correlated with the degree of inflammation found in muscle biopsies of patients with PM or with the clinical and laboratory parameters of these patients.

Although this was a retrospective study, data were collected from a database previously filed and standardised for all patients, making the information reliable. Moreover, although PM is a rare disease, we used strict inclusion and exclusion criteria to obtain a representative sample in this study.

All of the patients analysed in this study fulfilled the Bohan and Peter criteria (1, 2); however, these criteria do not allow for a clear distinction from other muscle diseases such as muscular dystrophy and inclusion body myositis. To improve the specificity of the criteria, we reevaluated all of the muscle biopsies and excluded any cases with muscle biopsies containing rimmed vacuoles.

Clinical and laboratory findings, such as constitutional symptoms, dysphagia, dysphonia, the degree of muscle weakness and the, serum muscle enzyme levels were not correlated with the presence and degree of inflammatory infiltrates found in the muscle biopsies. It is possible to have a muscle biopsy showing mild inflammatory infiltrates in patients with PM with extensive clinical and laboratory involvement, and the opposite trend is also possible. This dissociation between histologic findings and clinical and laboratory data could be because the inflammation found in muscle biopsies is often focal.

For giant cell arteritis, a systemic idiopathic vasculitis, there is not likely a correlation between the previous use of CS and the temporal artery histologic features (12-18). A case report showed the presence of inflammatory infiltrates in a temporal artery biopsy in a patient with giant cell arteritis who had taken prednisone 30–60 mg/day for six months (12). Other authors observed that the CS does not affect the inflammatory infiltrate after weeks (13) or years of use (14). One study showed that the use of CS might reduce the inflammatory infiltrates in temporal artery biopsies within one week of using CS (15). However, Breuer *et al*. (18) found no association between the histological features of the temporal artery and the clinical characteristics and outcomes of patients with giant cell arteritis.

There is indirect evidence of signs of inflammation in muscle biopsies from patients with idiopathic inflammatory myopathies who are already taking CS and/or immunosuppressant agents (5, 6). In a study of 35 patients with juvenile dermatomyositis, 9 (25.7%) received CS before the muscle biopsy (5), and five of these patients showed inflammatory infiltrates in the muscle biopsies. Adams *et al*. (19) reported a patient with juvenile PM with chronic use of several immunosuppressant agents who had evidence of oedema and fat muscle replacements on magnetic resonance imaging and evidence of inflammatory infiltrates in muscle biopsies. Tomasová *et al*. (9) evaluated the role of magnetic resonance imaging in the evaluation of patients with PM and dermatomyositis. These authors found inflammatory infiltrates in muscle biopsies in areas that appeared to be normal on magnetic resonance imaging. That study reported a significant decrease in the intensity of oedema observed on magnetic resonance, however, no significant histological changes of inflammatory infiltrates were found in the muscle biopsies in patients taking CS.

Our data are important for the clinical management of patients with suspected PM with muscle weakness in the limbs and increased serum levels of muscle enzymes. Although there is no scientific evidence to date, the introduction of CS therapy has been postponed until after the muscle biopsy. Muscle biopsies have been avoided in patients already taking CS because of the fear that there will not be inflammation on the biopsy. The CS use in patients with idiopathic inflammatory myopathies is relevant to improve the quality of life, to decrease muscular and other organs damages, to diminish the high cardiovascular comorbidities (20) and to increase the long-term survival of these patients (21, 22).

One previous study examined the effects of conventional immunosuppressant treatment on muscle histopathology (23). Treatment resulted in signifi-

cant reduction in the total number of macrophages (CD68 positive cells). By contrast, resident tissue macrophages (CD163 postiive cells) did not decrease significantly after treatment (23). Therefore, although the data from our large sample show that the presence and intensity of inflammatory infiltrate were similar in untreated and CS treated patients, additional studies are necessary to characterise the phenotype and distribution of these inflammatory cells found in the muscle biopsies exposed to CS.

Our data show that if clinical and laboratory indications of PM are present, it is reasonable to perform a muscle biopsy to confirm the inflammatory myopathy, even in patients who received previous CS treatment.

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