The effect of prior corticosteroid use in muscle biopsies from patients with dermatomyositis

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Abstract

Objective

The aim of this study was to evaluate the effect of prior corticosteroid (CS) use on the presence of inflammatory infiltrates (InI) in muscle biopsies from dermatomyositis (DM).

Methods

Sixty-five muscle biopsy samples were obtained at the time of DM diagnosis. The patients were divided into the following three groups according to the degree of the InI present in the muscle biopsies: (I) minimal InI present only in an interstitial area (endomysium, perimysium) or in a perivascular area; (II) moderate InI in one or two areas of the interstitium or of the perivascular area; and (III) moderate InI throughout the interstitium or intense inflammation in at least one area of the interstitium or of the perivascular area.

Results

All groups (I=17, II=16 and III=32) were comparable regarding the patient age at the time of the muscle biopsy, gender, ethnicity distribution, time interval between the muscle biopsy and the symptom onset, clinical manifestations, degree of muscle weakness, autoantibodies and serum muscle enzyme measurements ($p<0.05$). The median (interquartile) duration of CS use [7 (0–60), 6 (0–105) and 14 (0–30) days in groups I, II and III, respectively] and the median cumulative CS dose used [560 (0–2100), 1005 (0–2850) and 875 (0–2850) mg] were similar between the groups ($p>0.05$).

Conclusion

Previous CS use did not influence the presence or the degree of inflammatory infiltrates found in muscle biopsies in DM with clinical and laboratory disease activity. Therefore, muscle biopsies should be performed in this population, including patients currently undergoing CS therapy.

Key words

corticosteroids, dermatomyositis, inflammatory cells, muscle biopsy.

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Introduction

Dermatomyositis (DM) is an idiopathic inflammatory myopathy characterised by a subacute onset of symmetric limb muscle weakness with typical cutaneous lesions, such as heliotrope rash and/or Gottron's papules (1-5).

The treatment of DM is based on immunosuppressants (IS) and corticosteroid (CS) (6). The early introduction of such medications, particularly CS, might enable faster and more effective control of the disease activity of DM, leading to minimisation of morbidity and mortality.

However, the early introduction of CS might immediately interfere with the inflammatory process in the muscle tissues of patients with DM. Therefore, physicians have been postponing the introduction of drug therapy until after a muscle biopsy is performed to avoid possibly obscuring the histological diagnosis. In contrast, these biopsies have been also avoided in patients on CS therapy because this drug might obscure the more suggestive signs of inflammatory myopathies, and a 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 (7-10). Corroborating to these study, one study has shown recentely that polymyositis (PM) patients on extended CS therapy had inflammatory cell infiltration in muscle biopsies (10). On the other hand, another study showed a decreased presence of inflammatory cells after pulse therapy with intravenous methylprednisolone (11).

Thus, to define the effect of previous CS treatment on inflammatory cell infiltrations in muscle biopsy specimens, this study systematically reviewed the treatment regimens and histological muscle biopsies of patients with DM. Moreover, possible correlations between inflammatory cell infiltration in muscle biopsies and the severity of DM disease activity were also analysed.

Methods

This retrospective study to investigate the clinical manifestations of progressive symmetrical muscle weakness of limbs associated with cutaneous lesions (heliotrope rash and/or Gottron's papules) – confirmed by physicians – and high serum levels of skeletal muscle enzymes *(i.e.* creatine phosphokinase and aldolase) initially included 112 adult patients admitted to our tertiary centre from January of 2000 to January of 2014. Moreover, 68 of the 112 patients had electromyography results suggesting a pure inflammatory myopathy. None of our patients had neoplasia, overlapping systemic autoimmune diseases, chronic infections, family history of (neuro)muscle diseases or previous use of statins or fibrates.

Sixty-five out of 112 patients had muscle biopsies with signs of myopathy (fibres with regeneration, necrosis, degeneration, connective tissue alterations and/or different degrees of inflammatory cell infiltration), with or without the presence of perifascicular atrophy. Six cases were excluded from the study, since the muscle biopsy had not any morphological myopathy features. Thus, a total of 65 patients fulfilled the Bohan and Peter criteria and underwent analysis by muscle biopsy (1, 2).

The study was approved by the local Research Ethics Committee of our Institution.

The data were collected from our parameterised and standardised electronic medical records of these 65 patients. The following information was collected:

a) Demographic data, as follows: gender, age at the onset of DM, ethnicity, the time duration between the onset of the diagnosis and symptoms of DM;
b) Clinical manifestations, as follows: constitutional symptoms, cutaneous, gastrointestinal, cardiorespiratory and musculoskeletal involvement prior to the muscle biopsy. The limb muscle strength was graded according to the following Medical Research Council criteria; grade 0: absence of muscle contraction; grade I: slight signs of contractility; grade II: movements of normal amplitude, however, not against gravity; grade III: normal range of motion against gravity; grade IV: full mobility against gravity and a degree of resistance; and grade V: complete mo-

bility and strong resistance against the action of gravity (12);

c) Laboratory data: serum levels of muscle enzymes [creatine kinase (normal range: 24–173 U/L), aldolase (1.0–7.5 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 and anti-Mi-2 antibodies were determined with a commercially available line blot test kit (Myositis Profile Euroline Blot test kit, Euroimmun, Lübeck, Germany) according to a previously described method (13).

d) Treatment: the dose and duration of CS (prednisone or pulse therapy with intravenous methylprednisolone) treatment and/or IS prior to the muscle biopsy.

Muscle biopsies were performed routinely at the time of the DM 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 this study, we re-analysed the muscle biopsies by haematoxylin and eosin (H&E) staining. Each muscle biopsy specimen was coded and analysed separately by two investigators (S.K.S. and J.J.N.), who were blinded to the patient data. When a discrepancy was noted, the specimen was reviewed by both investigators to reach a consensus.

The following parameters were assessed semi-quantitatively as minimal, moderate or intense: fibre features (the presence of fibber degeneration, regeneration or necrosis); increased connective tissue (endomysial and/or perimysial); the presence of perifascicular atrophy; and the degree of inflammatory cell infiltration in the perimysial, endomysial and/or perivascular areas. Then, the patients with DM were classified semi-quantitatively into the following three groups (I, II and III) according to the presence of inflammatory cell infiltrates in the muscle biopsy specimens:

- Group I: minimal presence of inflammatory cell infiltrates restricted to one interstitial muscle biopsy area (endomysial, perimysial or perivascular);
- Group II: moderate presence of inflammatory cell infiltrates in one or two muscle biopsy interstitial areas;
- Group III: 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.

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 ± standard deviation (SD) for the continuous variables or as percentages (%) for the categorical variables. The median (25^{th}–75^{th} interquartile) was calculated for the continuous variables that were not normally distributed. Parameter comparisons among the three groups (I, II and III) were made using ANOVA or Kruskall Wallis test for the continuous variables. The analyses were performed with SPSS 15.0 statistics software (Chicago, USA). All values of $p<0.05$ were considered significant.

Results

Sixty-five patients with DM were evaluated and divided into three groups (I, II and III) based on the findings of the degree of inflammatory infiltrates in muscle biopsies (Table I).

Similarly to groups I and II, the histological findings of group III had a higher frequency of necrotic fibres, hyaline, basophilic and necrotic fibres associated with inflammatory cell infiltrations, predominantly in the perimysial and perivascular areas. Additionally, the group III present-

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

Parameters	Group I (n=17)	Group II (n=16)	Group III (n=32)	*p*-value
Age at disease onset (years)	42.6±16.0	40.7±17.7	44.3±14.0	0.747
Female gender	11 (64.7)	12 (75.0)	22 (68.7)	0.812
White ethnicity	14 (82.4)	12 (75.0)	28 (84.4)	0.729
Time between diagnosis and symptoms (months)	6.0 (3.0-9.0)	3.0 (2.0-6.5)	4.0 (2.0-6.5)	0.404
Constitutional symptoms	13 (76.5)	11 (68.8)	25 (78.1)	0.771
Dysphagia	10 (58.8)	7 (43.8)	20 (62.5)	0.458
Dysphonia	5 (29.4)	3 (18.8)	11 (34.4)	0.533
Articular involvement	11 (64.7)	8 (50.0)	11 (34.4)	0.533
Pulmonary involvement	5 (29.4)	7 (43.8)	10 (31.3)	0.623
Cutaneous involvement				
Heliotrope rash	15 (88.2)	13 (81.3)	27 (84.4)	0.856
Gottron's papules	16 (94.1)	15 (93.8)	28 (87.5)	0.669
Ulcers	2 (11.8)	2 (12.5)	4 (12.5)	0.997
Vasculitis	5 (29.4)	2 (12.5)	4 (12.5)	0.279
Photosensitivity	15 (88.2)	11 (68.8)	21 (65.6)	0.227
V-sign	10 (58.8)	5 (31.3)	11 (34.4)	0.179
Shawl sign	10 (58.8)	5 (31.3)	11 (34.4)	0.179
Muscle strength				
Upper limbs				
Grade V	1 (5.9)	0	1 (3.1)	-
Grade IV	12 (70.6)	11 (68.8)	15 (46.9)	0.174
Grade III	4 (23.5)	5 (31.3)	13 (40.6)	0.469
Grade II	0	0	3 (9.4)	-
Lower limbs				
Grade V	2 (11.8)	1 (6.3)	3 (9.4)	0.860
Grade IV	11 (64.7)	11 (68.8)	11 (34.4)	0.126
Grade III	4 (23.5)	4 (25.0)	14 (43.8)	0.250
Grade II	0	0	4 (12.5)	-
Antinuclear antibody	12 (70.6)	13 (81.3)	19 (59.4)	0.251
Anti-Jo1 antibody	1 (5.9)	4 (25.0)	3 (9.4)	0.247
Anti-Mi-2 antibody	2 (11.8)	2 (12.5)	8 (25.0)	0.381
Creatinine phosphokinase (U/L)	1238 (420-4786)	22586 (706-6389)	3629 (784-11171)	0.436
Aldolase (U/L)	20 (7.8-49.5)	23.4 (10.6-45.0)	33.4 (13.5-82.1)	0.626

Results expressed as a percentage (%), mean ± standard deviation, median (25^{th}–75^{th} interquartile).

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

Corticosteroid	Group I (n=17)	Group II (n=16)	Group III (n=32)	*p*-value
Prednisolone				
Current use (at muscle biopsy)	10 (58.8)	11 (68.8)	23 (71.9)	0.645
Time (days)	7 (0-60)	6 (0-105)	14 (0-30)	0.944
Cumulative dose (mg)	560 (0-2100)	1005 (0-5130)	875 (0-2850)	0.750
Methylprednisolone*	1 (5.9)	3 (18.8)	5 (16.6)	0.519

*Intravenous pulse therapy (dose: 1.5-5 g). Results expressed as a percentage (%) or the median (25^{th}–75^{th} interquartile).

ed the largest area of thickened endomysial and perimysial tissues.

Perifascicular atrophy was observed in 11.8%, 18.8% and 53.1% of the samples in groups I, II and III, respectively. The degree of the perifascicular atrophy was more evident in group III.

The three groups were alike regarding the demographic features and the time interval between the diagnosis of DM established by muscle biopsy and the onset of symptoms ($p>0.05$) (Table I).

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

More than one-half of the patients in each group were using CS at the time of the muscle biopsy (58.8%, 68.8% and 71.9% in groups I, II and III, respectively), as shown in Table II. The duration of CS use and the cumulative CS dose were similar among the groups ($p>0.05$). All patients without CS at the time of the muscle biopsy were naïve to this drug and to any IS.

One patient from group I had received pulse therapy with intravenous methylprednisolone, whereas three and five patients from groups II and III, respectively, had received this drug prior to muscle biopsy (Table II).

The maximum duration of CS use by patients in each group was 365, 575 and 1825 days, respectively, in groups I, II and III, and the maximum cumulative CS doses were 7.2, 21.6 and 8.1 g, respectively.

In addition to CS, five patients had also received IS prior to muscle biopsy:

i) Group I: one patient - parenteral cyclophosphamide 0.7 g/m^2 body surface for one month;
   one patient - methotrexate 15 mg/week for one year;
ii) Group II: no patients;
iii) Group III: one patient - azathioprine 2 mg/kg/day and intravenous human immunoglobulin 1 g/kg/day, two days, for two weeks;
   one patient - methotrexate 15 mg/week for 3 months;
   one patient - intravenous human immunoglobulin 1 g/kg/day, two days, for one month.

Discussion

The previous use of CS was not correlated with the degree of inflammation found in muscle biopsy of patients with DM or with the clinical and laboratory parameters of these patients.

Although the present study is retrospective, the analysed parameters are reliable as the data were collected from a database filed out systematically and standardised for all the patients. Additionally, rigorous inclusion and exclusion criteria were applied to select patients with defined DM.

Six patients were excluded from the study, since there were apparentely no morphologic myopathy characteristics or inflammatory cell infiltrations in muscle biopsies. This exclusion criteria were relevant to allow us to work only with patients who fulfilled all the Bohan and Peter criteria of DM and determine the real impact of the CS / IS in the biopsies with myopathic findings.

In our study, constitutional symptoms, degree of muscle weakness, dysphagia, dysphonia, autoantibodies and the serum muscle enzyme levels were not correlated with presence and degree of inflammatory infiltrates found in the muscle biopsies. The lack of correlation between the degree of histopathological and clinical parameters has been reported previously (9, 14, 15). Therefore, it is possible for a muscle biopsy to show mild inflammatory infiltrates in patients with DM with extensive clinical and laboratory involvement; additionally, the opposite trend is possible. This dissociation between histologic findings and clinical and laboratory data may result because, for instance, inflammation in muscle biopsies is frequently focal. Other hypothesis is that CS could decrease significantly the inflammatory infiltrates in muscle biopsies, but not the expression of some cytokines (for instance, IL-1α, ICAM-1 and VCAM-1) in the capillaries of muscle (14). These cytokines could be envolved in the persistent symptoms as muscle weakness (14).

One recent study of our group involving patients with PM also has shown that the previous CS use did not influence the presence or the degree of inflammatory infiltrates found in muscle biopsies with clinical and laboratory PM activity. Interestingly, even one clinically impaired PM patient who had received 10.8 g of CS for 270 days showed significant inflammatory cell infiltration in muscle biopsy (10). Similarly, in the present study, one DM patient presented intense inflammatory cell infiltrations in muscle biopsy even after being treated with 8.1 g CS for 1825 days.

There are other evidences of signs of inflammation in muscle biopsies from patients with idiopathic inflammatory myopathies after treatment of CS and/or IS agents (5, 6). In a study of juvenile DM, 25.7% received CS before the muscle biopsy (5), and approximately half these patients showed inflammatory infiltrates in the muscle biopsies. Additionally, Adams *et al*. (16) reported a patient with juvenile PM with chronic use of a number of IS agents who presented oedema and fat muscle replacement on magnetic resonance imaging and evidence of inflammatory infiltrates in muscle biopsy.

Moreover, previous study also reported similar results of persistency of inflam-

matory findings on muscle biospy concerning chronic use of IS (17). However, the IS treatment has resulted in significant reduction in the total number of macrophages (CD68 positive cells), but the resident tissue macrophages (CD163 positive cells) have not decreased significantly after treatment (17). Analogously, although the data from our large number of patients with DM have shown that the presence and intensity of inflammatory infiltrates were comparable in untreated and CS treated individuals, additional studies are necessary to characterise the phenotype and distribution of these inflammatory cells in muscle biopsies exposed to CS.

Our data are important for the clinical management of patients with suspected DM 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 currently taking CS with the fear to miss the diagnosis due to lack of inflammatory infiltration in the biopsy.

As limitations of the present study, we did not characterise the phenotype and distribution of the inflammatory cells found in the muscle biopsies exposed to CS. Second, the major histocompatibility complex (MHC) class I staining was also not performed.

However, our present results highlight that a muscle biopsy to confirm inflammatory myopathy is allowed even in DM patients who had received previous CS treatment.

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