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.
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 recently 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 (creatinine 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 fibre 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 (25th–75th 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-

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (n=17)</th>
<th>Group II (n=16)</th>
<th>Group III (n=32)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at disease onset (years)</td>
<td>42.6±16.0</td>
<td>40.7±17.7</td>
<td>44.3±14.0</td>
<td>0.747</td>
</tr>
<tr>
<td>Female gender</td>
<td>11 (64.7)</td>
<td>12 (75.0)</td>
<td>22 (68.7)</td>
<td>0.812</td>
</tr>
<tr>
<td>White ethnicity</td>
<td>14 (82.4)</td>
<td>12 (75.0)</td>
<td>28 (84.4)</td>
<td>0.729</td>
</tr>
<tr>
<td>Time between diagnosis and symptoms (months)</td>
<td>6.0 (3.0-9.0)</td>
<td>3.0 (2.0-6.5)</td>
<td>4.0 (2.0-6.5)</td>
<td>0.404</td>
</tr>
<tr>
<td>Constitutional symptoms</td>
<td>13 (76.5)</td>
<td>11 (68.8)</td>
<td>25 (78.1)</td>
<td>0.771</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>10 (58.8)</td>
<td>7 (43.8)</td>
<td>20 (62.5)</td>
<td>0.458</td>
</tr>
<tr>
<td>Dysphonia</td>
<td>5 (29.4)</td>
<td>3 (18.8)</td>
<td>11 (34.4)</td>
<td>0.533</td>
</tr>
<tr>
<td>Articular involvement</td>
<td>11 (64.7)</td>
<td>8 (50.0)</td>
<td>11 (34.4)</td>
<td>0.533</td>
</tr>
<tr>
<td>Pulmonary involvement</td>
<td>5 (29.4)</td>
<td>7 (43.8)</td>
<td>10 (31.3)</td>
<td>0.623</td>
</tr>
</tbody>
</table>

### Muscle strength

<table>
<thead>
<tr>
<th>Upper limbs</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade V</td>
<td>1 (5.9)</td>
<td>1 (3.1)</td>
<td></td>
</tr>
<tr>
<td>Grade IV</td>
<td>12 (70.6)</td>
<td>11 (68.8)</td>
<td>15 (46.9)</td>
</tr>
<tr>
<td>Grade III</td>
<td>4 (23.5)</td>
<td>5 (31.3)</td>
<td>13 (40.6)</td>
</tr>
<tr>
<td>Grade II</td>
<td>0</td>
<td>0</td>
<td>3 (9.4)</td>
</tr>
<tr>
<td>Lower limbs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade V</td>
<td>2 (11.8)</td>
<td>1 (6.3)</td>
<td>3 (9.4)</td>
</tr>
<tr>
<td>Grade IV</td>
<td>11 (64.7)</td>
<td>11 (68.8)</td>
<td>11 (34.4)</td>
</tr>
<tr>
<td>Grade III</td>
<td>4 (23.5)</td>
<td>4 (25.0)</td>
<td>14 (43.8)</td>
</tr>
<tr>
<td>Grade II</td>
<td>0</td>
<td>0</td>
<td>4 (12.5)</td>
</tr>
<tr>
<td>Antinuclear antibody</td>
<td>12 (70.6)</td>
<td>13 (81.3)</td>
<td>19 (59.4)</td>
</tr>
<tr>
<td>Anti-Jo1 antibody</td>
<td>1 (5.9)</td>
<td>4 (25.0)</td>
<td>3 (9.4)</td>
</tr>
<tr>
<td>Anti-Mi-2 antibody</td>
<td>2 (11.8)</td>
<td>2 (12.5)</td>
<td>8 (25.0)</td>
</tr>
<tr>
<td>Creatine phosphokinase (U/L)</td>
<td>1238 (421–4786)</td>
<td>22586 (706-6389)</td>
<td>3629 (784-11171)</td>
</tr>
<tr>
<td>Aldolase (U/L)</td>
<td>20 (7.8-49.5)</td>
<td>23.4 (10.6-45.0)</td>
<td>33.4 (13.5-82.1)</td>
</tr>
</tbody>
</table>

Results expressed as a percentage (%), mean ± a standard deviation, median (25th–75th interquartile).
ed the largest area of thickened endomy-
sial and perimysial tissues. Perifascicular atrophy was observed in
11.8%, 18.8% and 53.1% of the sam-
ples 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 constitu-
tional symptoms early in the disease and
the presence of dysphagia, dyspho-
nia, lung and articular involvement, the
degree of muscle weakness, the pres-
ence 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, re-
spectively), 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 meth-
ylprednisolone, whereas three and five
patients from groups II and III, respec-
tively, 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 cumula-
tive 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:

<table>
<thead>
<tr>
<th>Corticosteroid</th>
<th>Group I (n=17)</th>
<th>Group II (n=16)</th>
<th>Group III (n=32)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisolone</td>
<td>Current use</td>
<td>10 (58.8)</td>
<td>11 (68.8)</td>
<td>23 (71.9)</td>
</tr>
<tr>
<td></td>
<td>Time (days)</td>
<td>7 (0-60)</td>
<td>6 (0-105)</td>
<td>14 (0-30)</td>
</tr>
<tr>
<td></td>
<td>Cumulative dose (mg)</td>
<td>560 (0-2100)</td>
<td>1005 (0-5130)</td>
<td>875 (0-2850)</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>1 (5.9)</td>
<td>3 (18.8)</td>
<td>5 (16.6)</td>
<td>0.519</td>
</tr>
</tbody>
</table>

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

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

i) Group I: one patient - parenteral cy-
clophosphamide 0.7 g/m² body sur-
face 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 im-
munoglobulin 1 g/kg/day, two days,
for one month.

### Discussion
The previous use of CS was not cor-
related 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 retro-
spective, the analysed parameters are
reliable as the data were collected from
a database filed out systematically and
standardised for all the patients. Ad-
ditionally, rigorous inclusion and ex-
clusion criteria were applied to select
patients with defined DM.
Six patients were excluded from the
study, since there were apparently no
morphologic myopathy characteristics or
inflammatory cell infiltrations in
muscle biopsies. This exclusion crite-
ria were relevant to allow us to work
only with patients who fulfilled all the
Bohan and Peter criteria of DM and de-
termine the real impact of the CS / IS in
the biopsies with myopathic findings.
In our study, constitutional symptoms,
degree of muscle weakness, dyspha-
gia, 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 correla-
tion between the degree of histopatho-
logical and clinical parameters has
been reported previously (9, 14, 15).
Therefore, it is possible for a muscle
biopsy to show mild inflammatory in-
filtrates in patients with DM with ex-
tensive clinical and laboratory involve-
ment; additionally, the opposite trend
is possible. This dissociation between histologic findings and clinical and
laboratory data may result because, for
instance, inflammation in muscle biop-
sies is frequently focal. Other hypo-
thes is is that CS could decrease signifi-
cantly the inflammatory infiltrates in
muscle biopsies, but not the expression
of some cytokines (for instance, IL-1α,
ICAM-1 and VCAM-1) in the capil-
laries 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 inflamma-
tory infiltrates found in muscle biopsies
with clinical and laboratory PM activ-
ity. Interestingly, even one clinically
impaired PM patient who had received
10.8 g of CS for 270 days showed sig-
ificant inflammatory cell infiltration
in muscle biopsy (10). Similarly, in the
present study, one DM patient present-
ed intense inflammatory cell infiltra-
tions 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 juve-
nile DM, 25.7% received CS before the
muscle biopsy (5), and approximately
half these patients showed inflamma-
tory 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 pre-
sented oedema and fat muscle replace-
ment on magnetic resonance imaging
and evidence of inflammatory infil-
trates in muscle biopsy.
Moreover, previous study also reported
similar results of persistency of inflam-
Dermatomyositis muscle biopsies / S.K. Shinjo et al.

Matomy findings on muscle biopsy 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.

References