REPORT

Anti-Jo-1 antibody-positive patients show a characteristic necrotizing perifascicular myositis

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Idiopathic inflammatory myopathies can be classified as polymyositis, dermatomyositis, immune-mediated necrotizing myopathy, sporadic inclusion body myositis or non-specific myositis. Anti-Jo-1 antibody-positive patients are assigned to either polymyositis or dermatomyositis suggesting overlapping pathological features. We aimed to determine if anti-Jo-1 antibody-positive myopathy has a specific morphological phenotype. In a series of 53 muscle biopsies of anti-Jo-1 antibody-positive patients, relevant descriptive criteria defining a characteristic morphological pattern were identified. They were tested in a second series of anti-Jo-1 antibody-positive patients and compared to 63 biopsies from patients suffering from other idiopathic inflammatory myopathies. In anti-Jo-1 antibody-positive patients, necrotic fibres, which strongly clustered in perifascicular regions, were frequently observed. Sarcolemmal complement deposition was detected specifically in perifascicular areas. Inflammation was mainly located in the perimysium and around vessels in 90.6%. Perimysial fragmentation was observed in 90% of cases. Major histocompatibility complex class I staining was diffusely positive, with a perifascicular reinforcement. Multivariate analysis showed that criteria defining perifascicular pathology: perifascicular necrosis, atrophy, and perimysial fragmentation allow the distinction of anti-Jo-1 antibody-positive patients, among patients suffering from other idiopathic inflammatory myopathies. Anti-Jo-1 antibody-positive patients displayed perifascicular necrosis, whereas dermatomyositis patients exhibited perifascicular atrophy.

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**Abbreviations:** ASS = anti-synthetase syndrome; DM = dermatomyositis; IIM = idiopathic inflammatory myopathy; IMNM = immune mediated necrotizing myopathy; MHC = major histocompatibility complex

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### Introduction

To date, idiopathic inflammatory myopathies (IIMs) are classified in five categories: polymyositis, dermatomyositis (DM), immune-mediated necrotizing myopathy (IMNM), sporadic inclusion body myositis and non-specific myositis (Hoogendijk et al., 2004). Pathological analysis of skeletal muscle biopsies represents an important element for classification of IIM (Hoogendijk et al., 2004). In addition to the five histological patterns currently associated with the five main categories of idiopathic inflammatory myopathies, there are >15 myositis-specific autoantibodies. Some of these autoantibodies, including anti-tRNA-synthetase autoantibodies, define more homogeneous groups of patients than current histopathological classifications. Similarly, Love et al. (1991) and later, Koenig et al. (2007) proposed a clinicoserological classification including a new category: overlap myositis, for patients harbouring myositis-specific autoantibodies. The anti-histidyl-tRNA synthetase antibody (anti-Jo-1) is the most common myositis-specific autoantibody, encountered in 20–30% of patients with IIM (Brouwer et al., 2001). Anti-aminoacyl-tRNA synthetase antibody-positive patients suffer from myositis associated with specific extramuscular manifestations, which include interstitial lung disease, arthritis, Raynaud’s phenomenon and mechanic’s hands, defining the relatively homogenous interstitial lung disease, arthritis, Raynaud’s phenomenon with specific extramuscular manifestations, which include antibody-positive patients suffer from myositis associated with a specific morphological phenotype, as the extramuscular manifestations suggest a distinct disease.

### Materials and methods

#### Patients

First, muscle biopsies of 53 Jo-1 patients (French series) were analysed retroactively. Anti-Jo-1 antibodies had been identified using two different standard methods (Luminex-100 system; Luminex, or the ENA-LISA, Biomedical Diagnostics). Clinical signs of patients and ancillary data were documented by two of the co-authors (B.H., O.B.) using the same methodology as described previously (Hervier et al., 2012; Stanciu et al., 2012).

In a second part of the study, muscle biopsy specimens of 19 Jo-1 patients (German series) were analysed. Patients were identified based on similar diagnostic criteria as those in the French series.

In addition, the IIM control groups consisted of muscle biopsies from patients tested negative for anti-Jo-1, anti-PL7 and -PL12 antibodies (n = 63), suffering from dermatomyositis (n = 20), immune-mediated necrotizing myopathy (IMNM; n = 21) and sporadic inclusion body myositis (n = 22). All patients fulfilled ENMC criteria of dermatomyositis and IMNM (Hoogendijk et al., 2004). Sporadic inclusion body myositis was diagnosed according to the Hilton-Jones criteria (Benveniste and Hilton-Jones, 2010).

According to the principles of the Declaration of Helsinki, informed consent was obtained from all patients and the Charité ethics committee approved the study (EA1/204/11). All patients gave informed consent to participate in the study, and approval was obtained from the French institutional review board (AC 2012 1724).

#### Histological analysis

Seven-micrometre cryostat sections of muscle biopsies were used for the study. Stains and immunophenotyping of inflammatory cells were performed as previously described (Preusse et al., 2012; Allenbach et al., 2015), using an automated slide staining system (BenchMark XT, Ventana Medical Systems). The term ‘perimysial’ describes alterations in the connective tissue domain in between the fascicles, and the term ‘perifascicular’ describes alterations in the muscle fibre domain at the very edge (two penultimate layers) of the muscle fascicles (Wedderburn et al., 2007).

Double immunofluorescence of utrophin and C5b-9 was performed with the same protocol and repeated with a second primary antibody and an appropriate secondary antibody.

For vascular assessment, endothelial cells were stained with utrophin in Jo-1 patients (n = 20), dermatomyositis patients (n = 19) and compared to patients with normal muscle biopsies (n = 7).

#### Electron microscopy

Ultrastructural analysis was performed by the same methodology as described previously (Preusse et al., 2012).

#### Histological quantifications

First, all slides from the French series were retroactively analysed. Presence or absence of each item is mentioned. Necrosis
was defined by pale and/or hyalinized staining by haematoxylin and eosin. Regenerating fibres were identified by increased basophilia with haematoxylin and eosin staining as well as large vesicular nuclei and by foetal myosin or NCAM staining. Additionally, a semiquantitative analysis was performed for assessment of inflammation, fibre necrosis, and C5b-9 deposition on capillaries: inflammation and necrosis were scored 0: absent, 1: mild and 2: prominent; C5b-9 capillary deposits score was 0 if no deposit, 1 if ≤5; 2 if 6–10, and 3 if >10 capillaries showed deposits. The localization (perimysial, endomysial or perifascicular) of lymphocytes and necrosis was recorded. The perifascicular region was defined as the two penultimate myofibre layers at the periphery of fascicles, and perifascicular necrosis was considered if more than two-thirds of the total number of necrotic fibres occurred in perifascicular regions.

The German series of Jo-1 patients and the abovementioned control groups were analysed blinded to diagnosis.

For vascular assessment, 10 randomly selected areas were photographed at an original magnification of 200-fold using the Olympus DP25 camera. For each picture the number of whole muscle fibre sections and capillaries were manually counted, and the capillary:fibre ratio was calculated (Estruch et al., 1992).

**Statistical analysis**

The Mann-Whitney or the Kruskal-Wallis tests were used to compare histological characteristics between inflammatory myopathy groups, when appropriate. A P-value <0.05 was considered significant.

Features associated with presence of Jo-1 antibodies were studied, using a model of logistic regression. The parameters listed in Supplementary Table 1 were used as independent variables in a logistic model with a stepwise backward selection as well. The diagnostic performance of the score was illustrated by the receiver operating characteristic curve (SAS 9.3 software).

**Results**

**Characteristics of the patients**

Characteristics of Jo-1 patients (French, n = 53) are reported in Table 1. As expected, all Jo-1 patients presented a myopathy with at least two of the following features: proximal weakness, myalgia, myogenic changes on electromyogram and/or high creatine kinase levels. In the second group of Jo-1 patients (German n = 19), the mean age was 46.7 ± 15.5 years and 60% were female. In the IIM control groups, DM patients were 48.2 ± 18.4 years old, 84.2% female; IMNM patients were 57.6 ± 16.6 years old, 66.6% females; sporadic inclusion body myositis patients were 68.6 ± 5.1 years old, and 40% were female. DM patients were anti-Mi-2 antibody-positive in 27.2% of the cases and IMNM patients were either anti-signal recognition particle (SRP) (56%) or anti-3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR) (44%) auto-antibody-positive.

**Perifascicular fibre injury in Jo-1 patients**

The most salient feature of Jo-1 patients’ biopsies was presence of necrotic fibres in general (86.8% of patients had necrotic fibres), which strongly clustered in perifascicular regions (75.8%) (Fig. 1A). In line with the predominance of necrotic fibres in perifascicular regions, regenerating fibres were frequently observed (83%), mainly in the same area. Immune stainings using anti-CD56, anti-foetal myosin and anti-utrophin antibodies, confirmed the presence of regenerating fibres in perifascicular areas (Supplementary Fig. 1). Atrophic myofibres occurred frequently and clustered in perifascicular regions with conspicuous irregular distribution, as 58.8% of patients harboured this pattern. Sarcolemmal complement deposition was restricted to perifascicular fibres (75%) (Fig. 1B). Sarcolemmal complement deposition occurs in a subgroup of regenerating fibres in Jo-1 patients, whereas we did not observe substantial co-labelling in DM patients (Supplementary Fig. 1).

Major histocompatibility complex (MHC) class I staining was diffusely positive in 92.2%, with a perifascicular reinforcement in 58.8% of cases (Fig. 1C).

**Perimysial inflammation in Jo-1 patients**

Inflammation was present in 50 biopsies (94.3%); scores 1 and 2 were noted in 43.4% and 51% of cases, respectively. The infiltrate was mainly located in the perimysium and/or around vessels in 90.6%, with extension into the endomysium in 83.0% (Fig. 1A). Endomysial lymphocytes invading (43.4%) or surrounding (60.4%) non-necrotic fibres were present. Additionally, inflammation was associated with perimysial fragmentation, which was highlighted by alkaline phosphatase staining (90% of cases) (Fig. 1D), and by ultrastructural analysis illustrating localization in the perimysial fibrous tissue as well as swollen fibroblasts (Fig. 1E).

CD68+ macrophages were most abundant and detected in all cases, T cells were found in nearly all cases (CD4+ cells: 93%; and CD8+ cells 90.5%), while CD20+ B cells were observed in 62% of the cases.

Topographically, macrophages and CD8+ cells were found both in the endomysium, and perimysium (Fig. 1F). CD4+ cells were mainly seen in the perimysium and around vessels extending into the endomysium in 50%, whereas CD20+ B cells were nearly exclusively detected in the perimysium (Fig. 1F).

Together these results showed that Jo-1 patients exhibit perimysial and perifascicular pathology.
Compared to other IIM patients, Jo-1 patients harbour a distinct pattern of perifascicular injury

Having identified perifascicular necrosis and scattered atrophy to be associated with perimysial inflammation and fragmentation, we aimed to analyse these items in an independent series of IIM patients to test their specificity, including Jo-1, DM, IMNM, and sporadic inclusion body myositis patients.

Eleven relevant descriptive items were analysed (Supplementary Table 1) defining the perifascicular pattern of actual diagnostic criteria mainly led to the diagnosis of IMNM was not made due to presence of at least one exclusion criterion (such as presence of C5b-9 deposits of fibres or significant inflammatory infiltrates) (Hoogendijk et al., 2004). Thus, strict application of actual diagnostic criteria mainly led to the diagnosis of dermatomyositis because the region of injury is similar in Jo-1 and DM patients.

### Jo-1 patients and their pattern of perifascicular injury fall into the category of dermatomyositis

According to ENMC criteria (Hoogendijk et al., 2004), diagnosis of dermatomyositis was achieved in 77.3% of cases among our two Jo-1 series. Classical dermatomyositis was diagnosed in 26.4% of the cases (20.8% ‘definite DM’, and 5.6% ‘probable DM’), and 50.9% of patients were diagnosed as possible ‘DM sine dermatitis’, as they did not harbour skin lesions compatible with dermatomyositis rash (Table 1). The remaining 22.6% of patients were diagnosed as polymyositis (3.7% as definite and 18.8% as probable). Of note, all ‘polymyositis patients’ harboured necrosis, but a diagnosis of IMNM was not made due to presence of at least one exclusion criterion (such as presence of C5b-9 deposits of fibres or significant inflammatory infiltrates) (Hoogendijk et al., 2004). Thus, strict application of actual diagnostic criteria mainly led to the diagnosis of dermatomyositis because the region of injury is similar in Jo-1 and DM patients.

### Dermatomyositis-like vascular pathology also occurs in Jo-1 patients

To differentiate DM and Jo-1 patients, we asked whether Jo-1 patients also showed signs of vasculopathy commonly observed in DM (Hoogendijk et al., 2004). Vasculopathy in Jo-1 patients was evidenced by C5b-9 deposits on capillaries, rarefaction of capillaries or presence of endothelial tubuloreticular inclusions. Capillaries showed C5b-9 deposits adjacent to the perimysium in 84% of the Jo-1 cases, but mainly with a low intensity (score 3, 32%; score 2, 26%; and score 1, 26%, respectively) (Fig. 2A). Compared to dermatomyositis, this percentage was not significantly different from the one observed in patients with dermatomyositis (95%, P = 0.4). Conspicuous capillaries were observed in both groups, but more frequently in Jo-1 patients (100% and 84% in DM and Jo-1 patients, respectively, P = 0.01) (Fig. 2B). A rarefaction of capillaries (Fig. 2C), evidenced by low capillary:fibre ratio, was observed in Jo-1 patients (1.13 ± 0.23), but the difference was not significant compared to that in DM patients (1.03 ± 0.31; P = 0.13). This ratio in patients with normal muscle biopsy was higher (1.58 ± 0.26) compared to both Jo-1 and DM patients (P < 0.001) (Fig. 2D). Furthermore, ultrastructural analysis revealed tubuloreticular inclusions in endothelial cells in 47% of Jo-1 patients versus 100% in DM patients (P = 0.0001) (Fig. 2E). Together, these results show that vascular changes commonly observed in DM also occurred in Jo-1, differences were, however, only detectable using ultrastructural analysis.

### Table 1 Clinical characteristics of 53 Jo-1 patients (French series)

<table>
<thead>
<tr>
<th>Patients</th>
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<tbody>
<tr>
<td>Mean age at ASS diagnosis (year) n = 53</td>
<td>45 ± 14</td>
</tr>
<tr>
<td>Gender (females) n = 53</td>
<td>58.5% (31)</td>
</tr>
<tr>
<td>Muscle involvement</td>
<td></td>
</tr>
<tr>
<td>Myalgia n = 45</td>
<td>73% (33)</td>
</tr>
<tr>
<td>Muscle weakness* n = 53</td>
<td>77% (41)</td>
</tr>
<tr>
<td>Mean creatine kinase (IU/ml) n = 53</td>
<td>7950 ± 10 460</td>
</tr>
<tr>
<td>Myogenic syndrome n = 34</td>
<td>76% (26)</td>
</tr>
<tr>
<td>Intersitial lung disease n = 53</td>
<td>79% (42)</td>
</tr>
<tr>
<td>Polyrhegalgia/polyarthritus n = 51</td>
<td>82% (42)</td>
</tr>
<tr>
<td>Raynau's phenomenon n = 50</td>
<td>34% (17)</td>
</tr>
<tr>
<td>Mechanics’ hands n = 50</td>
<td>26% (13)</td>
</tr>
<tr>
<td>Other skin lesions* n = 51</td>
<td>27% (14)</td>
</tr>
<tr>
<td>Autoantibodies</td>
<td></td>
</tr>
<tr>
<td>Jo-1 n = 53</td>
<td>100% (53)</td>
</tr>
<tr>
<td>PL7 or PL12 n = 53</td>
<td>0% (0)</td>
</tr>
<tr>
<td>TRIM-21/Ro52 n = 41</td>
<td>56% (23)</td>
</tr>
<tr>
<td>Ro60/SSA n = 39</td>
<td>28% (11)</td>
</tr>
<tr>
<td>La/SSB n = 39</td>
<td>5% (2)</td>
</tr>
<tr>
<td>RNP n = 39</td>
<td>5% (2)</td>
</tr>
</tbody>
</table>

n = number of patients with available information in the databases; Ab = antibody.

*Defined as Medical Research Council sum score ≤4 for at least ≥1 muscle group evaluated by manual muscle testing.

*Including skin rash compatible with a DM rash, sclerodactyly and/or digital ulcers.
Jo-1 patients harbour a ‘perifascicular necrotizing’ myositis and DM patients harbour a ‘perifascicular atrophy’ myositis

To further investigate whether perifascicular pathology observed in DM patients differs from that in Jo-1 patients, we compared the characteristics of the perifascicular pathology in both groups. We selected the following relevant criteria to define perifascicular pathology: necrotic fibres, atrophic fibres, sarcolemmal positivity for C5b-9, MHC class I immunostaining mainly occurring in perifascicular regions and perimysial fragmentation or perimysial inflammatory infiltrates. Of note, univariate analysis showed that these criteria were significantly more frequent in Jo-1 and
DM patients compared to the others IIMs (data not shown).

Multivariate analysis showed that Jo-1 patients displayed perifascicular necrosis more frequently (79% versus 35%, \( P = 0.007 \)) whereas DM patients exhibited perifascicular atrophy more frequently (85% versus 63%, \( P = 0.04 \)) (Table 2). Characteristics of muscle inflammation were not significantly different between both groups.

**Discussion**

In this study comprising 72 Jo-1 patients and 63 patients with other IIMs, we show that Jo-1 patients harbour a very characteristic ‘necrotizing perifascicular myositis’ whereas DM is characterized by a ‘perifascicular atrophy myositis’.

Importantly, the region of interest in necrotizing perifascicular myositis is the perifascicular area, a phenomenon that was first highlighted by Pestronk (2011) who coined the term of immune myopathy with perifascicular pathology (IMPP) comprising Jo-1 myopathies, and IMPP patients with skin involvement are frequently classified as having dermatomyositis (Pestronk, 2011). This may explain why Jo-1 patients have been classified as DM patients.

However, the extramuscular phenotype of Jo-1 patients is clearly different from the one in DM patients. In particular,
presence of interstitial lung disease in ~80% of Jo-1 patients, is present only in a minority of DM cases (Connors et al., 2010). Furthermore, ‘DM patients’ with interstitial lung disease frequently encompass an important proportion of ASS patients (Hamaguchi et al., 2011). In addition, the characteristic heliotrope rash observed in DM is not a defining element of ASS, where mechanics’ hands are a cornerstone. As a result, only 25% of Jo-1 patients in this series showed skin lesions ‘compatible’ with DM. Moreover, DM patients generally harbour an increased risk of malignancy compared to other IIMs and the general population (András et al., 2008), whereas we and others have previously reported that this is not the case for Jo-1 patients (Chinoy et al., 2007; Hervier et al., 2012). Of note, some Jo-1 patients could have also been diagnosed as having IMNM (Christopher-Stine et al., 2010; Preusse et al., 2012; Stenzel et al., 2012). However, necrotic fibres in IMNM are diffusely distributed, and inflammation per se is less prominent. At variance with others (Mozaifar et al., 2000), we showed that inflammation and its topography are a conspicuous diagnostic element.

The characteristic pattern we observed in Jo-1 patients adds further data to a recent study addressing this topic. The authors reported that HLA-DR myofibre expression is more frequently observed in patients suffering from ASS (82.8%) compared to that in DM patients (27.6%) (Aouizerate et al., 2014).

To conclude, we observed a uniform and characteristic myopathological pattern in the largest series of Jo-1 patients to date, which is in line with the well-established homogenous clinical phenotype. This entity shares the same topography of pathology with DM, which may have led to previously classifying it as DM. However, it is the perifascicular necrosis, defining the myopathology in Jo-1 patients. This implies that Jo-1 patients should no longer be considered having dermatomyositis or polymyositis, and morphological diagnostic criteria ought to be adjusted accordingly.

### Table 2 Perifascicular pattern in Jo-1 and DM patients

<table>
<thead>
<tr>
<th>Pathologic features</th>
<th>DM (n = 20)</th>
<th>Jo-1 (n = 19)</th>
</tr>
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<tbody>
<tr>
<td>Myofibre necrosis in perifascicular regions</td>
<td>7 (35%)</td>
<td>15 (79%)*</td>
</tr>
<tr>
<td>Myofibre atrophy in perifascicular regions</td>
<td>17 (85%)*</td>
<td>12 (63%)</td>
</tr>
<tr>
<td>Perimysial fragmentation</td>
<td>9 (45%)</td>
<td>14 (74%)</td>
</tr>
<tr>
<td>Perimysial inflammatory infiltrates</td>
<td>20 (100%)</td>
<td>19 (100%)</td>
</tr>
<tr>
<td>HLA enhancement in perifascicular regions</td>
<td>17 (85%)</td>
<td>15 (79%)</td>
</tr>
<tr>
<td>Sarcolemmal positivity for C5b-9 in perifascicular regions</td>
<td>10 (50%)</td>
<td>14 (74%)</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01 calculated after multivariate analysis.

### Funding
Société Française de Médecine Interne, Association Française contre les Myopathies.

### Supplementary material
Supplementary material is available at Brain online.

### References
Connors GR, Christopher-Stine L, Odds CV, Danoff SK. Interstitial lung disease associated with the idiopathic inflammatory myopathies: what progress has been made in the past 35 years? Chest 2010; 138: 1464–74.
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