# Novel assessment tools to evaluate clinical and laboratory responses in a subset of patients enrolled in the Rituximab in Myositis trial

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## Abstract

### Objective

*We aimed to assess changes in myositis core set measures and ancillary clinical and laboratory data from the National Institutes of Health's subset of patients enrolled in the Rituximab in Myositis trial.*

### Methods

*Eighteen patients (5 dermatomyositis, 8 polymyositis, 5 juvenile dermatomyositis) completed more in-depth testing of muscle strength and cutaneous assessments, patient-reported outcomes, and laboratory tests before and after administration of rituximab. Percentage change in individual measures and in the definitions of improvement (DOIs) and standardised response means were examined over 44 weeks.*

### Results

*Core set activity measures improved by 18–70% from weeks 0–44 and were sensitive to change. Fifteen patients met the DOI at week 44, 9 patients met a DOI 50% response, and 4 met a DOI 70% response. Muscle strength and function measures were more sensitive to change than cutaneous assessments. Constitutional, gastrointestinal, and pulmonary systems improved 44–70%. Patient-reported outcomes improved up to 28%. CD20+ B cells were depleted in the periphery, but B cell depletion was not associated with clinical improvement at week 16.*

### Conclusion

*This subset of patients had high rates of clinical response to rituximab, similar to patients in the overall trial. Most measures were responsive, and muscle strength had a greater degree of change than cutaneous assessments. Several novel assessment tools, including measures of strength and function, extra-muscular organ activity, fatigue, and health-related quality of life, are promising for use in future myositis trials. Further study of B cell-depleting therapies in myositis, particularly in treatment-naïve patients, is warranted.*

**Key words**

rituximab, juvenile dermatomyositis, dermatomyositis, polymyositis, outcome assessment, biological therapy, idiopathic inflammatory myopathies, CD20 depletion, clinical trial

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*Received on November 25, 2013; accepted in revised form on March 20, 2014.*



*Funding: this study was supported in part by the National Institute of Environmental Health Sciences, National Institutes of Health Intramural Research program (LGR, ALY, RV, AVJ, FWM), the National Cancer Institute Intramural Research Program (MLT, HHK), and an interagency agreement with the National Institute of Arthritis and Musculoskeletal and Skin Diseases (LGR, FWM). Rituximab was supplied by Genentech, San Francisco, California, USA.*

*Competing interests: none declared.*

## Introduction

Rituximab, a chimeric monoclonal antibody that targets the CD20 protein on the surface of B cells, is approved for treating non-Hodgkin's lymphoma, rheumatoid arthritis (RA), and granulomatosis with polyangiitis and microscopic polyangiitis (1, 2). Rituximab may be useful for treating other autoimmune diseases (3, 4) by decreasing the levels of autoantibodies by depleting B cells and autoreactive plasma cells (5), increasing regulatory T cells, and resetting tolerogenic mechanisms (4).

Rituximab may be useful for treating myositis, a systemic autoimmune disease characterised by chronic muscle inflammation and proximal muscle weakness. In the first open-label trials of rituximab for dermatomyositis (DM), muscle strength and function improved after B cell depletion, and a few treatment-refractory juvenile DM patients showed marked improvement (6-8). A meta-analysis found that 72% of 51 treatment-refractory myositis patients had decreased disease activity and improved strength and function with rituximab (9). The Rituximab in Myositis (RIM) trial was a randomised placebo-phase design conducted in 200 treatment-refractory patients with adult DM or polymyositis (PM) or juvenile DM. The RIM trial determined the response to rituximab based on time to improvement in 3 of 6 core set measures, as validated by the International Myositis Assessment and Clinical Studies Group (IMACS) (10, 11); 83% of patients had a clinical response, although there was no difference in response time between treatment groups (11).

There is limited information about the effects of rituximab on other organ systems in myositis. An open-label trial suggested that DM skin symptoms do not respond to rituximab despite improvement in muscle strength (12). Because there is also limited information regarding several novel assessment tools in myositis, we performed additional, in-depth assessments of the 18 patients in the RIM trial who enrolled at the National Institutes of Health (NIH) Clinical Center, including detailed muscle and skin assessments, patient-reported outcomes (PROs), and laboratory studies that were adjunct to those completed in the full RIM trial.

## Patients and methods

### *Patients*

Eighteen patients in the multicentre RIM trial (11) enrolled through the NIH Clinical Center in Bethesda, Maryland, USA. These patients met study criteria, received all doses of rituximab, and completed assessment measures. The study was approved by the National Institute of Diabetes and Digestive and Kidney Diseases/ National Institute of Arthritis and Musculoskeletal and Skin Diseases institutional review board, and all patients signed informed consent in accordance with the Helsinki Declaration.

Eight patients had PM, 5 had DM, and 5 had juvenile DM, all meeting probable or definite Bohan and Peter criteria (13, 14). Median disease duration was 68 months [interquartile range (IQR) 42–101 months]. Adult patients had a median age of 37.9 years [IQR 30–56 years], and the four paediatric patients had a median age of 12.3 years [IQR 9.9–16.9 years]. Thirteen patients (72%) were female, and 7 each were white or black, 3 were Hispanic, and 1 was Asian. Sixteen patients were taking prednisone (median daily dose 20.0 mg or 0.28 mg/kg/day). Other concomitant therapies for myositis continued during the trial included methotrexate (n=14 patients), azathioprine (n=8), hydroxychloroquine (n=4), mycophenolate mofetil (n=2), and cyclosporine (n=1). Three patients had anti-Jo-1 autoantibodies, 4 had anti-signal recognition particle, 1 had anti-Mi-2, 2 with juvenile DM had anti-p155/140, and 1 had probable anti-MJ autoantibodies, by immunoprecipitation testing (15).

### *Study design*

Patients were randomly assigned to receive rituximab at weeks 0 and 1 and placebo at weeks 8 and 9 (rituximab-early group) or placebo at weeks 0 and 1 followed by rituximab at weeks 8 and 9 (rituximab-late group) (11). Six patients (2 each with PM, DM, and juvenile DM) were in the rituximab-early group, and 12 patients (6 PM, 3 DM, 3 juvenile DM) were in the rituximab-

late group. Each myositis core set activity measure (16) was assessed monthly over 44 weeks, with trial endpoints as described by Oddis *et al*. (11). We used the Myositis Disease Activity Assessment Tool (MDAAT), which includes visual analog scales (VAS), the Myositis Intention-to-Treat Activity Index (MITAX) for 6 different systems (constitutional, cutaneous, skeletal, gastrointestinal, pulmonary, and cardiovascular), and the Extra-muscular Global Activity Score (0–10 cm VAS) (17). The primary definition of improvement (DOI) was met if, at 2 consecutive visits, there was ≥20% improvement in 3 of 6 core set activity measures, with ≤2 core set measures (but not muscle strength) demonstrating >25% worsening (10). We also determined how many patients had ≥50% and ≥70% improvement in the DOI, analogous to moderate and major clinical responses to therapy for RA and juvenile DM (18, 19).

*Additional assessments*

At weeks 0, 16, and 44, NIH patients underwent additional clinical evaluations not performed in the larger Rituximab in Myositis trial. These included assessment of muscle strength by 24-muscle-group manual muscle testing (MMT, total and proximal scores) (20) and by fixed-frame isometric dynamometry to assess the MMT-8 muscle groups (16), and muscle function by the Childhood Myositis Assessment Scale (CMAS) (16) and gait analysis (21). Skin activity was assessed by dermatologists (MLT, HHK) and a rheumatologist (LGR) using the Cutaneous Disease Activity Severity Index (CDASI) and the Disease Activity Score (DAS) (16). Muscle and skin assessors were blinded to treatment status. PROs included the SF-36 for adults and Child Health Questionnaire Parent Form-50 (CHQ-PF50) as quality-of-life measures (16), the Paediatric Quality-of-Life Index (PedsQL), Multidimensional Fatigue Scale and Fatigue Severity Scale as measures of fatigue (22, 23), the Dermatology Life Quality Index (DLQI) (16), and for adult patients, the dyspnea questionnaire of the Human Activity Profile (24). Parents of paediatric patients completed the PRO questionnaires.

Lymphocyte flow cytometry was performed by standard methods (25). Absolute lymphocyte counts were performed on the same blood sample, and B cell depletion was defined to be <5 peripheral cells/μL (26). Directly conjugated reagents included anti-CD20, -CD38, and -CD5 (Becton Dickinson); anti-CD45 and -CD27 (eBioscience, San Diego, CA); and anti-CD19 (Invitrogen, Carlsbad, CA).

Short-tau inversion recovery (STIR) axial thigh magnetic resonance imaging (MRI) was analysed by visual semi-quantitative scoring (27). Three compartments (anterior, medial, and posterior) were scored on a 0–5-point Likert scale reflecting the degree of oedema or fatty infiltration, and these were summed to create total disease activity and damage MRI scores.

*Statistical analysis*

SigmaStat 3.1 (Systat Software Inc., San Jose, CA) and GraphPad Prism 5.02 (GraphPad Software Inc., San Diego, CA) were used. Median value and IQR were determined. The median percentage change in scores was calculated for weeks 0–16, 16–44, and 0–44. Time point values were compared using the Wilcoxon signed-rank test. For $CD20^+$ B cells, the percentage change for each time interval was compared between the early- and late-treatment groups using the Mann-Whitney test. The standardised response mean (SRM) was used to examine the responsiveness of different outcomes, calculated as the average of the absolute difference between weeks 0 and 44 divided by the standard deviation of the difference (28). To compare the responsiveness between two different measures, the SRM for each patient was compared in a pairwise manner using the Wilcoxon signed-rank test.

## Results

*Changes in myositis core set measures*

After rituximab, all myositis core set measures of disease activity improved by 18–70% from week 0 to 44 (Table I). In examining the threshold for clinically important change (29), we noted that 16 of 18 patients improved by ≥20% in physician and patient/parent global activity assessment, and 15 patients improved ≥20% in extra-muscular activity; 14 and 10 patients improved ≥15% in physical function (assessed using the Health Assessment Questionnaire [HAQ]/Childhood HAQ [CHAQ]) and muscle strength (MMT-8), respectively; and 11 patients improved ≥30% in serum muscle enzyme levels. Based on the SRM values, the core set measures were very sensitive to change (Table I). Creatine kinase level, the serum muscle enzyme that was most often highest at baseline, was less sensitive to change than the other core set measures ($p$=0.03–0.001). The (C)HAQ was less sensitive to change than the Physician Global Activity ($p$<0.001) and the Extra-muscular Global Activity ($p$=0.008) scores. No difference in the response by treatment group was detected.

Eight (44%) of the 18 patients met the DOI by week 16, and 15 (83%) met the DOI by week 44, similar to the overall RIM trial results (11). Using the original trial endpoint, 9 (50%) of the 18 NIH patients met a DOI 50% response, and 4 patients (22%) met a DOI 70% response. No patient had a complete clinical response or entered remission (30).

*Muscle versus cutaneous assessments*

In the 10 adult and juvenile DM patients, we compared responses in muscle and skin (Table II). Their muscle strength and functional measures improved throughout the trial, with median improvements of 17–64% for weeks 0–44 (Table II). Most muscle measures were very responsive and had comparable sensitivity to change. The Muscle MITAX was less sensitive to change (SRM 0.7) than the Muscle VAS portion of the MDAAT or than the MMT-8, Total MMT (SRM 2.1), and Proximal MMT scores, based on their SRMs ($p$=0.010–0.037). The (C)HAQ and CMAS were less responsive than the Proximal MMT and MMT-8 scores ($p$=0.027) (Table II). The mean gait velocity decreased only 9% from weeks 0–44 (data not shown).

For cutaneous assessments in DM patients (Table II), only the DLQI improved at week 44, by a median of 43% ($p$=0.047). Other skin assessments did not improve significantly, but they showed a moderate to high degree of

**Table I.** Changes in myositis core set activity measures after rituximab therapy for 18 patients enrolled in the RIM trial at the NIH.

| Core set measure [potential range] | Baseline value median [IQR] | Week 0-16 median % improvement | Week 16-44 median % improvement | Week 0-44 median % improvement | SRM¥ week 0-44 |
|---|---|---|---|---|---|
| Physician Global Activity (VAS) [0-10 cm] | 4.5 [3.5, 5.1] | 15[b] | 33[c] | 46[c] | 2.0 |
| Patient/Parent Global Activity (VAS) [0-10 cm] | 5.8 [5.0, 7.0] | 12[a] | 45[c] | 46[c] | 1.6 |
| Manual Muscle Test-8 [0-80] | 51.0 [47.0, 61.0] | 8[c] | 9[c] | 18[c] | 1.5 |
| [Childhood] Health Assessment Questionnaire* [0-3] | 1.4 [1.3, 2.3] | 18[a] | 17[c] | 36[c] | 1.2 |
| MDAAT Extra-muscular Global Activity VAS [0-10 cm] | 3.2 [3.0, 3.5] | 18 | 38[c] | 70[c] | 1.9 |
| Most abnormal lab enzyme‖ | N/A | 29 | 26 | 58[b] | N/A |
| Creatine kinase [38-252 U/L] | 705.0 [198.5, 2875.0] | 39 | 25 | 52[b] | 0.6 |
| Aldolase [1-6 U/L] | 16.4 [5.5, 42.9] | 6 | 21[a] | 41[b] | 0.8 |
| Lactate dehydrogenase [113-226 U/L] | 268.0 [218.3, 437.0] | 8 | 15[a] | 17[a] | 0.8 |

RIM: Rituximab in Myositis; NIH: National Institutes of Health; IQR: interquartile range; SRM: standardised response mean; VAS: visual analogue scale; MDAAT: Myositis Disease Activity Assessment Tool.

¥ Creatine kinase was less sensitive to change than the other core set activity measures (SRM $p<0.05$). The (C)HAQ was also less responsive than Physician Global Activity (SRM $p<0.001$) and the MDAAT Extra-muscular Global Activity VAS (SRM $p<0.01$).

*The Childhood and Adult Health Assessment Questionnaire scores are combined.

‖ Eleven patients had creatine kinase as their most abnormal lab enzyme, 4 had aldolase, 2 had lactate dehydrogenase, and 1 had alanine aminotransferase.

[a] $p<0.05$, [b] $p<0.005$, [c] $p<0.001$ *vs*. baseline.

**Table II.** Changes in muscle and skin assessments after rituximab therapy for 10 adult and juvenile dermatomyositis patients enrolled in the RIM trial at the NIH.

| Measure [potential range] | Baseline value median [IQR] | Week 0-16 median % change¥ | Week 16-44 median % change¥ | Week 0-44 median % change¥ | SRM* week 0-44 |
|---|---|---|---|---|---|
| *Muscle* | | | | | |
| MMT-8 Score [0-80] | 54.5 [48.5, 61.0] | 21[a] | 9[b] | 28[c] | 2.2 |
| MMT Proximal Score [0-160] | 101.0 [94.3, 123.8] | 19[b] | 9[b] | 31[b] | 2.2 |
| QMT-8 [0-250 lb.] | 61.8 [47.7, 96.9] | 7 | 31[b] | 64[b] | 1.3 |
| MDAAT Muscular VAS [0-10 cm] | 4.8 [2.9, 5.3] | 30[b] | 26[a] | 48[c] | 1.5 |
| DAS Muscle [0-11] | 8.0 [6.8, 9.0] | 6 | 11 | 17[a] | 1.4 |
| CMAS [0-52] | 34.0 [19.0, 38.3] | 11[a] | 21[a] | 26[a] | 1.2 |
| (C)HAQ [0-3] | 1.4 [1.1, 2.3] | 22[a] | 36[a] | 50[a] | 1.2 |
| *Skin* | | | | | |
| CDASI [0-168] | 17.5 [2.0, 29.0] | 1 | 30 | 17 | 0.9 |
| DLQI [0-30] | 4.5 [1.0, 7.0] | -20 | 72 | 43[a] | 0.6 |
| MDAAT Cutaneous VAS [0-10 cm] | 3.4 [2.0, 4.5] | 17 | 24 | 28 | 1.1 |
| DAS Skin [0-9] | 6.0 [2.0, 7.0] | 0 | 0 | 0 | 1.0 |

RIM: Rituximab in Myositis; NIH: National Institutes of Health; IQR: interquartile range; SRM: standardised response mean; MMT-8: manual muscle testing of 8 muscle groups (20); MMT Proximal: manual muscle testing proximal score (39); QMT-8: quantitative muscle testing or isometric dynamometry average score for the 8 muscle groups included in the MMT-8 (20); DAS: Disease Activity Score; CMAS: Childhood Myositis Assessment Scale; (C)HAQ: (Childhood) Health Assessment Questionnaire; CDASI: Cutaneous Dermatomyositis Disease Area and Severity Index; DLQI: Dermatology Life Quality Index; MDAAT: Myositis Disease Activity Assessment Tool; VAS: visual analogue scale.

¥Positive values indicate improvement, whereas negative values indicate worsening.

*The Proximal and Total MMT scores were more sensitive to change than the CDASI, DLQI, and the DAS Skin ($p=0.05$–$0.006$). The Proximal MMT score was also more responsive than the MDAAT Cutaneous VAS ($p<0.05$); and the MDAAT Muscular VAS was more responsive than the DLQI ($p=0.023$ for difference in their SRMs).

[a] $p<0.05$, [b] $p<0.01$, [c] $p<0.005$ *vs*. baseline.

responsiveness based on their SRMs. The Cutaneous MITAX was less sensitive to change (SRM 0.5) than the Cutaneous VAS of the MDAAT (SRM 1.1, $p=0.037$). The responsiveness of other cutaneous measures was comparable (Table II).

The muscle assessments were more sensitive to change than skin assessments, based on a pairwise comparison of their SRMs (Table II). The Total and Proximal MMT scores were more responsive than the CDASI, DLQI, and the DAS Skin scores ($p=0.05$–$0.006$); the Proximal MMT score was also more responsive than the MDAAT Cutaneous VAS ($p<0.05$); and the MDAAT Muscle VAS was more responsive than the DLQI ($p=0.023$). There were no significant differences in responsiveness between the other muscle and cutaneous measures.

The STIR MRI semi-quantitative muscle oedema signal in the gluteal, anterior, medial, and posterior regions improved by a median of 20% from weeks 16–44 ($p=0.005$). Other MRI subscores, including subcutaneous and fascial oedema and T1 muscle damage, did not change.

*Extra-muscular assessment*

The MDAAT extra-muscular organ

VAS scores improved from weeks 0–44 in the Constitutional (median improvement 65%), Gastrointestinal (median improvement 70%), Pulmonary (median improvement 44%), and Extra-muscular Global Activity subscales (median improvement 70%, $p<0.001$ for each) (Fig. 1). The Skeletal VAS scores improved only from weeks 0–16 (median improvement 56%, $p<0.01$), and the Cardiovascular VAS scores improved only from weeks 16–44 (median improvement 10%, $p=0.01$) (not shown). The Constitutional VAS score (SRM 2.7) was more responsive than the Constitutional MITAX score (SRM 1.2, $p=0.0004$). There were no differences in the responsiveness of the VAS *versus* MITAX scores for the other extra-muscular organ systems or for the Extra-muscular Global Activity scores and no differences in response by treatment group (data not shown).

The Constitutional (SRM 2.7) and Extra-muscular VAS (SRM 1.9) scores were more responsive than those for each of the other systems assessed by the MDAAT (SRM 0.9–1.1, $p=0.02$–$0.002$ for differences in SRMs of the VAS scores). The Muscle VAS score (SRM 1.9) was more responsive than the scores for the skeletal, gastrointestinal, pulmonary, and cardiovascular systems ($p=0.01$–$0.003$) and did not differ from the overall Extra-muscular Global VAS score. The Extra-muscular MITAX score (SRM 1.6) was more responsive than the MITAX scores for each organ system (SRM 0.5–1.2, $p=0.02$–$0.002$, not shown).

*Patient-reported outcomes*

In the 14 adult DM and PM patients, the SF-36 physical and mental summary scores improved, with greater change in the physical than mental summary score (Table III). Improvements in other subdomains of the SF-36 from weeks 0–44 included the physical function score (median improvement 100%, $p=0.03$), role physical score (median improvement 50%, $p=0.034$), general health score (median improvement 28%, $p=0.021$), mental health score (median improvement 13%, $p=0.045$), and vitality score (median improvement 40%, $p=0.006$) (not shown).

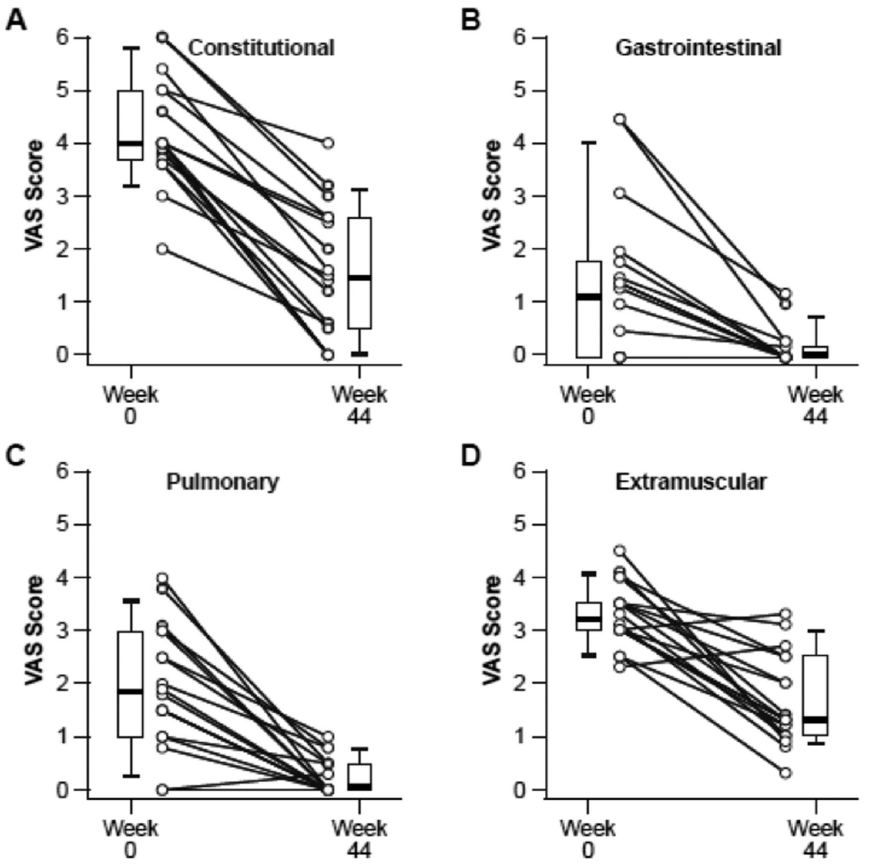


**Fig. 1.** Myositis Disease Activity Assessment Tool Visual Analogue Scale (VAS) scores in selected extra-muscular systems. Individual patient data are displayed. Data for the cutaneous system for adult and juvenile dermatomyositis patients are in Table II. (**A**) Constitutional VAS, standardised response mean (SRM) = 2.7. (**B**) Gastrointestinal VAS, SRM = 0.9. (**C**) Pulmonary VAS, SRM = 1.1. (**D**) Extra-muscular VAS, SRM = 1.9.

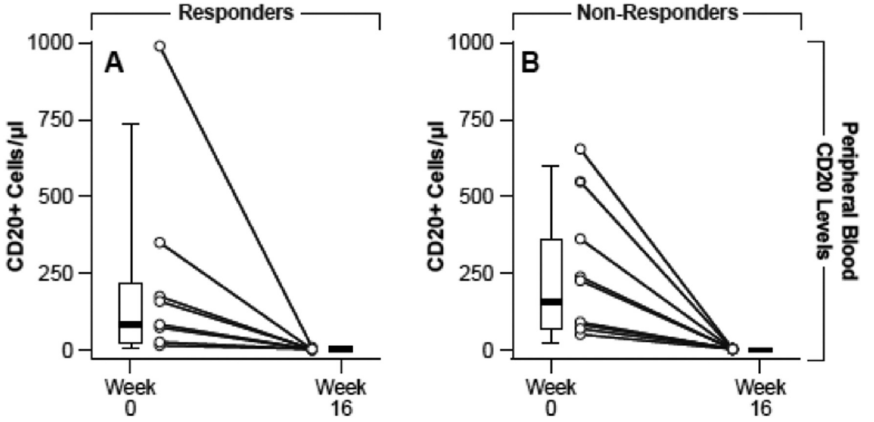


**Fig. 2.** CD20+ B cells in the peripheral blood and muscle before and after rituximab. **A**. Peripheral blood CD20+ B cells in 8 patients meeting the definition of improvement (DOI) criteria at week 16 (Responders). Median CD20 counts were 120 cells/µl at week 0 and 0.5 cells/µL at week 16. **B**. Peripheral blood CD20+ B cells in 9 patients without a clinical response at week 16. Median CD20 counts were 225 cells/µL at week 0 and 0 cells/µL at week 16.

The PedsQL Multidimensional Fatigue Scale Total score and General Fatigue subscale improved 25% and 71%, respectively (Table III). The PedsQL General Fatigue subscale was more responsive than the total score from weeks 0–44 ($p<0.01$). The General and Sleep subscales of the PedsQL

**Table III.** Patient-reported outcomes after rituximab therapy for 18 patients enrolled in the RIM trial at the NIH.

| Measure[¥] [potential range] | Baseline median [IQR] | Week 0-16 median % change | Week 16-44 median % change | Week 0-44 median % change | SRM* |
|---|---|---|---|---|---|
| SF-36 Physical Sum Score [0-100] | 29.1 [23.5, 35.7] | 9 | 11 | 28[a] | 1.2 |
| SF-36 Mental Sum Score [0-100] | 49.2 [34.4, 57.0] | 0 | 5[a] | 12[a] | 1.2 |
| CHQ Physical Sum Score [0-100] | 28.0 [22.6, 31.5] | -4 | 29 | 25 | 1.4 |
| CHQ Psychosocial Sum Score [0-100] | 51.7 [45.1, 59.3] | 10 | -5 | 3 | 0.9 |
| PedsQLFatigue – Total Score [0-100] | 54.9 [30.6, 61.1] | 14[a] | 12[a] | 25[b] | 1.0 |
| Fatigue Severity Scale [9-63] | 50.5 [37.0, 63.0] | 0 | 11 | 3 | 1.0 |
| Dyspnea Scale [0-3] | 2.0 [1.5, 2.8] | 7 | 8 | 14 | 1.0 |

*Abbreviations per Table I.* SF -36: Short Form 36 Health Survey; CHQ: Child Health Questionnaire Parent Form 50; PedsQLFatigue: Paediatric Quality of Life Index Multidimensional Fatigue Scale; Dyspnea Scale: from the Human Activity Profile (24).

[¥]The SF-36 was completed by adult patients (n=14), and the CHQ was completed by parents of children (n=4). All 18 patients, or in the case of the children, their parents, completed the PedsQL, the Fatigue Severity Scale, and the DLQI. The Dyspnea Scale was completed by adult patients only. Positive values indicate improvement, and negative values represent worsening.

*The SRM did not differ between measures.

[a] $p<0.05$, [b] $p<0.001$ *vs.* baseline.

Fatigue measure also improved from weeks 0–44 (median 71% and 31%, respectively), whereas the Cognitive subscale did not change. The Fatigue Severity scale and Dyspnea subscale of the Human Activity Profile did not change. The SRMs for PedsQL Total Fatigue and Fatigue Severity scales were similar, and there were no differences when compared with other PROs (SF-36, Dyspnea scale), but the subscales of the PedsQL Fatigue measure were more responsive than the DLQI ($p$=0.01–0.05). There were no differences by treatment group.

*Peripheral blood lymphocytes and lack of correlation of B cell depletion with clinical response*

Rituximab depleted $CD20^+$ B cells in all but one patient. All patients in the rituximab-early treatment group depleted peripheral blood $CD20^+$ B cells at weeks 8 and 16. By week 44, peripheral blood B cells returned in 3 patients (50%) in the rituximab-early group (range 51–451 cells/μL). In the rituximab-late group, 11 patients (92%) depleted B cells at week 16. By week 44, peripheral blood B cells returned in 8 patients (73%) in the rituximab-late group (range 6–296 cells/μL). Similar trends were observed by treatment group in $CD19^+$ cells, $CD20^+CD5^+$ (B1-like B cells), and $CD20^+CD27^+$ (memory B cells). No significant changes were observed in T cell markers ($CD3^+$ or $CD5^+$ cells), T cell activation markers ($CD3^+HLA^-DR^+$, or $CD3^+CD25^+$ cells), NK cells (NK or NK-T cells), T cell subsets ($CD4^+CD3^+$, $CD8^+CD3^+$, double-negative T cells), or naïve and memory T cells ($CD4^+CD45RO^+$, $CD4^+CD45RA^+$, and $CD3^+CD8^+CD57^+$ cells) (not shown).

Depletion of peripheral blood B cells did not correlate with clinical response at week 16, in that responders and non-responders (based on the DOI) both reduced $CD20^+$ B cells to a similar extent (Fig. 2A-B). Similar trends were observed for $CD20^+CD27^+$ B cells, except that 1 non-responder had an increase in memory B cells at week 16.

## Discussion

In this study we characterised the response to rituximab in many organ systems by using several different validated outcome assessments beyond those performed in the primary Rituximab in Myositis trial (16). Many patients improved throughout the trial by achieving minimally important clinical change in the core set measures (29) and in the DOI as a criterion of important clinical improvement (10). Using the original trial endpoint, 50% of our patients met a DOI 50% response and 22% met a DOI 70% response, suggesting a great degree of response to rituximab in a number of treatment-refractory patients (30).

We found that many measures of disease activity were very responsive after rituximab therapy. Among the core set activity measures, the Physician Global Activity, MMT, and Extra-muscular Global Activity scores were most sensitive to change. Among the muscle strength and functional assessments, the MMT scores and Muscle VAS score of the MDAAT were most responsive. Among the skin assessments, only the DLQI improved significantly. Among the extra-muscular scores, the Constitutional and Extra-muscular VAS scores were most responsive, and the PedsQL Fatigue scores markedly improved. These findings suggest that rituximab affected not only muscle strength and function, but also extra-muscular organs and PROs, particularly fatigue and health-related quality of life. Several assessments, including core set activity measures, extra-muscular organs, and the SF-36, were more responsive in this trial than in an etanercept trial of treatment-refractory adult DM patients (31). Several measures, including gait velocity and CDASI, were poorly responsive in both trials. Muscle oedema on MRI improved slightly after rituximab treatment. Measures of strength and function, extra-muscular organ activity, fatigue, and health-related quality of life, appear to be promising for use in future myositis trials.

In the present trial, in adult and juvenile DM patients, muscle strength measures were more sensitive to change and improved more than cutaneous measures after rituximab treatment. These data are consistent with the open-label trial of

rituximab in adult DM patients, where muscle strength improved significantly but skin activity did not (12). Despite relatively small samples sizes, these data suggest that rituximab had greater effects on muscle than on cutaneous disease activity in DM. As expected, rituximab depleted $CD20^+$ B cells but did not significantly change T cell counts in peripheral blood (32, 33). In RA, other synovial lineages, including T cells, macrophages, and fibroblast-like synoviocytes, were reduced by rituximab therapy (34, 35). The depletion of $CD20^+$ B cells or $CD20^+CD27^+$ memory B cells in the peripheral blood did not seem to correlate with clinical response. Inconsistent correlation with depletion of $CD20^+$ B cells in the peripheral blood and synovium and clinical response has also been reported in RA, although a stronger relationship appears to exist between depletion of memory B cells and clinical response in RA (33-36). Factors other than B cell depletion, including low expression of interferon pathway genes, that are predictive of clinical response in RA, may be important in determining response to rituximab (37). Clinical and autoantibody subgroups, which are important outcome predictors (38), also predict rituximab response in IIM (39).

The results of this analysis are limited as follows: only 18 patients underwent these detailed assessments and at limited time points (weeks 16 and 44). The sample size was not large enough to examine randomisation effects or differences between disease subgroups, and heterogeneity in phenotypes may have led to variability in responses. Several exploratory analyses were conducted, but given the multiple comparisons made, the findings would be prone to type I error, although this is preferred to avoid rejecting the null hypothesis too readily in exploratory analyses (40). Finally, although many measures were responsive, findings may be limited by subjects' anticipation of improvement, as all subjects received active therapy, and by the subjective nature of some of the assessments.

Responses to rituximab in this subset of patients enrolled in the RIM trial were similar to those in the overall trial (11). This detailed study of rituximab therapy found improvement in muscle, fatigue, and other extra-muscular organs but little response in the skin of myositis patients. Further study of B cell–depleting agents in myositis is needed, particularly in treatment-naïve patients. Several assessment tools appear to be sensitive to change, including certain measures of muscle strength and function, extra-muscular organ activity, and fatigue. These measures may be valuable in future, larger clinical trials testing new therapeutic agents for patients with myositis.

**Acknowledgements**

We thank the following individuals for assistance with patient coordination: Laura James-Newton, Yan Li, and Kathleen Gorman; Ellen Levy for quantitative muscle testing; Christopher J. Stanley and Karen Siegel for gait analysis; Dr Lawrence Yao for MRI scoring; and Dr Peter A. Lachenbruch for statistical consultation. We thank Dr Galen Joe for access to licenses of the SF-36 through the Department of Rehabilitation Medicine. We thank Dr Ann Reed, Ms Diane Koontz, and members of the RIM trial steering committee for their development, organisation, and support of the RIM trial. We thank Dr Mark Gourley, Maria Villalba, and Peter Lachenbruch for critical reading of the manuscript, and Drs Gourley and Paul Plotz for patient referrals.

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