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Predictors of Clinical Improvement in Rituximab-Treated Refractory Adult and Juvenile Dermatomyositis and Adult Polymyositis

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Objective. **To identify the clinical and laboratory predictors of clinical improvement in a cohort of myositis patients treated with rituximab.**

Methods. **We analyzed data for 195 patients with myositis (75 with adult polymyositis [PM], 72 with adult dermatomyositis [DM], and 48 with juvenile DM) in the Rituximab in Myositis trial. Clinical improvement was defined as 20% improvement in at least 3 of the following 6 core set measures of disease activity: physician's and patient's/parent's global assessment of disease activity, manual muscle testing, physical function, muscle enzymes, and extramuscular disease activity. We analyzed the association of the following baseline variables with improvement: myositis clinical subgroup, demographics, myositis damage, clinical and laboratory parameters, core set measures, rituximab treatment, and myositis autoantibodies (antisynthetase, anti–Mi-2, anti–signal recognition particle, anti–transcription intermediary factor 1γ [TIF-1γ], anti-MJ, other autoantibodies, and no autoantibodies). All measures were univariately assessed for association with improvement using time-to-event analyses. A multivariable time-dependent proportional hazards model was used to evaluate the association of individual predictive factors with improvement.**

Results. **In the final multivariable model, the presence of an antisynthetase, primarily anti–Jo-1 (hazard ratio [HR] 3.08, $P < 0.01$), anti–Mi-2 (HR 2.5, $P < 0.01$), or other autoantibody (HR 1.4, $P = 0.14$) predicted a shorter time to improvement compared to the absence of autoantibodies. A lower physician's global assessment of damage (HR 2.32, $P = 0.02$) and juvenile DM (versus adult myositis) (HR 2.45, $P = 0.01$) also predicted improvement. Unlike autoantibody status, the predictive effect of physician's global assessment of damage and juvenile DM diminished by week 20. Rituximab treatment did not affect these associations.**

Conclusion. **Our findings indicate that the presence of antisynthetase and anti–Mi-2 autoantibodies, juvenile DM subset, and lower disease damage strongly predict clinical improvement in patients with refractory myositis.**

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The idiopathic inflammatory myopathies (IIMs) are a group of acquired, heterogeneous, systemic connective tissue diseases (CTDs) that includes polymyositis (PM), adult dermatomyositis (DM), childhood myositis (predominantly juvenile DM), myositis associated with cancer or another CTD, and inclusion body myositis (IBM) (1,2). Over the last few decades, survival has improved in IIM, with patients experiencing less cumulative damage and better health-related quality of life. Despite an improvement in survival, our knowledge about clinical and serologic predictors of clinical improvement in IIM is limited by a lack of well-designed, long-term epidemiologic studies and clinical trials. IIM patients have heterogeneous features ranging from a mild rash to life-threatening muscle weakness or lung involvement. The disease course can be self-limited or may require long-term glucocorticoid treatment and multiple immunosuppressive medications. The response to immunosuppressive drugs is quite variable and current data do not allow the accurate prediction of clinical improvement, which poses a significant challenge to treating physicians as well as investigators.

The varying clinical features of myositis are closely linked to myositis autoantibodies, some of which may contribute to the pathogenesis of IIM (3). Although these autoantibodies provide useful prognostic information on patient outcomes (4–6), this relationship has not been established in prospective cohorts with uniform treatment. Previous evidence suggested that patients possessing anti–Mi-2 autoantibodies had a better prognosis, while patients with anti–signal recognition particle (anti-SRP) fared worse and those with antisynthetase autoantibodies had intermediate outcomes (6,7).

In addition, there is a paucity of literature regarding predictors of clinical improvement by IIM disease subgroups. IBM is associated with poor treatment responses, but studies differentiating responses between PM, DM, and juvenile DM are lacking (6). Treatment delay, muscle damage, and longer disease duration have also been shown to be associated with poor prognosis (7–10). However, published studies are limited by small sample sizes, retrospective design, and a limited assessment of prognostic factors.

The availability of targeted therapies and validated outcome measures (11–13) prompted the recently completed Rituximab in Myositis (RIM) trial that was designed to evaluate the safety and efficacy of B cell depletion in adult and pediatric myositis patients (14). Rituximab has been studied in a wide variety of autoimmune diseases, as B cells play a critical role in the initiation and propagation of the immune response and are specifically implicated in the pathogenesis of myositis (15). Since biologic agents are increasingly used to treat autoimmune diseases, it is important to elucidate the factors that predict a favorable outcome so that clinical trials can be designed with stratification of patients with a good likelihood and those with a poor likelihood of improvement. The aim of this study was to identify the clinical and laboratory predictors of clinical improvement in patients with refractory myositis treated with B cell depletion. This is the first comprehensive study in myositis to evaluate factors associated with clinical improvement in a large prospective cohort.

PATIENTS AND METHODS

Patients and definition of improvement. A total of 200 patients (76 with adult PM, 76 with adult DM, and 48 with juvenile DM) with refractory myositis (14) were treated with rituximab as part of a multicenter clinical trial (RIM) using a randomized placebo phase design (16). However, only 195 were enrolled for at least 2 weeks and available for analysis with regard to achieving the definition of improvement. Refractory myositis was defined as an intolerance of or an inadequate response to glucocorticoids and at least one other immunosuppressive agent. Patients were randomized to either a "rituximab early" arm (drug at weeks 0 and 1 and placebo at weeks 8 and 9) or a "rituximab late" arm (placebo at weeks 0 and 1 and drug at weeks 8 and 9), such that all patients received active drug at some point in the study.

The definition of improvement was the International Myositis Assessment and Clinical Studies group preliminary validated response (11) of a $\geq$20% improvement in 3 of any 6 core set measures (17), with no more than 2 core set measures worsening by $\geq$25% (which could not include manual muscle strength testing [MMT]). The primary end point was the time to achieve the definition of improvement at 2 consecutive time points. The 6 core set measures (17) for this trial were patient's (or parent's) global assessment of disease activity using a 10-cm visual analog scale (VAS), physician's global assessment of disease activity using a 10-cm VAS, the Health Assessment Questionnaire (HAQ) or Childhood HAQ (C-HAQ), serum muscle enzyme level (most abnormal of creatine kinase, aldolase, lactate dehydrogenase, alanine aminotransferase, or aspartate aminotransferase), global extramuscular disease activity (based on the investigator's composite assessment of disease activity on the constitutional, cutaneous, skeletal, gastrointestinal, pulmonary, and cardiac scales of the Myositis Disease Activity Assessment Tool [MDAAT]) (13), and MMT, assessed using a validated measure, the MMT-8 (18). The myositis core set measures that were assessed in determining the definition of improvement were collected at 14 visits over a 44-week period. Members of the RIM Study Group are shown in Appendix A.

Baseline predictor (independent) variables. Two of the authors (RA and CVO) selected a priori the baseline clinical, laboratory, and serologic variables that were evaluated for their potential to predict clinical improvement. Variable selection was based on clinical experience and a literature review of

Table 1. Baseline predictor variables analyzed for univariate analysis

Group	Variables*
Demographic features	Age at trial entry, age at diagnosis, sex, ethnicity, and disease duration
Myositis clinical subgroup	Polymyositis, dermatomyositis, or juvenile dermatomyositis
Laboratory parameters	Total IgM and IgG levels, hemoglobin, leukocyte count, platelet count, and serum creatinine
Autoantibody status†	Antisynthetase, anti–Mi-2, other autoantibodies, or no autoantibodies
Baseline myositis damage‡	Muscle damage, GI damage, pulmonary damage, and physician's global assessment of damage
Baseline myositis disease activity§	Skeletal (i.e., inflammatory arthritis), GI, pulmonary, and muscle disease activity
Myositis core set activity measures	1. Physician's global assessment of disease activity¶ 2. Patient's (or parent's) global assessment of disease activity¶ 3. Assessment of muscle strength using the MMT-8 score# 4. Assessment of physical function using the HAQ or C-HAQ 5. Muscle enzyme levels** 6. Global extramuscular disease activity (13)††
Baseline medication	Early versus late rituximab treatment arm, number of total failed immunosuppressive agents at trial entry, and baseline glucocorticoid dose (in prednisone equivalents)
Categorical baseline MDAAT variables	Dysphagia, arthritis, mechanic's hands, and active ILD‡‡
Categorical baseline MDI variables	Calcinosis, muscle atrophy, radiographic pulmonary fibrosis, and abnormal DLco or FEV_1
Other	Raynaud's phenomenon, clinical trial site

* HAQ = Health Assessment Questionnaire; C-HAQ = Childhood HAQ; FEV_1 = forced expiratory volume in 1 second.
† Patients were classified into 4 groups based on autoantibody status as described in Patients and Methods.
‡ Muscle damage, gastrointestinal (GI) damage, and pulmonary damage were measured using the Myositis Damage Index (MDI), a validated tool for assessing damage in muscle and extramuscular organ systems on a 10-cm visual analog scale (VAS) (42). The physician's global assessment of damage was measured on a 10-cm VAS.
§ Measured by the Myositis Disease Activity Assessment Tool (MDAAT), a validated measure for assessing physician-rated myositis and extramuscular disease activity on 10-cm VAS (13).
¶ Measured on a 10-cm VAS.
# Assessed using a validated measure, Manual Muscle Testing 8 (MMT-8) (18).
** Muscle enzyme levels were designated as times the upper limit of normal of the most abnormal muscle enzyme (of creatine kinase, aldolase, lactate dehydrogenase, alanine aminotransferase, or aspartate aminotransferase).
†† Measured using the MDAAT tool (10-cm VAS composite assessment of disease activity on the constitutional, cutaneous, skeletal, gastrointestinal, pulmonary, and cardiac scales) (13).
‡‡ Active interstitial lung disease (ILD) was defined as dyspnea, cough, parenchymal abnormalities on chest radiography or computed tomography, or pulmonary function tests showing a $\geq$10% change in forced vital capacity or diffusing capacity for carbon monoxide (DLco) (with the physician attributing the change to active reversible ILD).

previous studies (19–21). The variables selected for analysis are listed in Table 1.

Autoantibodies were detected using protein and RNA immunoprecipitation (IP) (14) and were classified into 4 groups: 1) myositis autoantibodies including the anti-aminoacyl–transfer RNA synthetases (anti–Jo-1, anti–PL-7, anti–PL-12, anti-KS, anti-OJ, and anti-EJ), anti–Mi-2, anti-SRP, anti–transcription intermediary factor 1γ (anti–TIF-1γ), and anti-MJ; 2) other known autoantibodies seen in myositis and/or other CTDs (anti–PM-Scl, anti–U1 RNP, anti-SSA/SSB, anti-Ku, anti-SAE, anti-U1/U2, and anticentromere antibody); 3) undefined autoantibodies (i.e., those that could not be definitively identified by IP), and 4) patients with no detectable autoantibodies. Since the Kaplan-Meier curves for the groups of patients with anti-SRP, anti–TIF-1γ, anti-MJ, other known autoantibodies, and undefined autoantibodies were overlapping and not significantly different from each other, these groups were consolidated and analyzed as one category, termed "patients with other autoantibodies." Thus, 4 autoantibody subsets emerged for the final statistical analysis: antisynthetase, anti–Mi-2, other autoantibodies, and no autoantibodies.

Statistical analysis. The baseline for this study was defined as week 0 of the RIM trial regardless of whether a patient was in the early or late treatment arm. As in the RIM trial, the primary outcome was the time to achieve the definition of improvement assessed in time-to-event analyses. All baseline variables were univariately assessed for association with time to achieve the definition of improvement. All univariate variables that had a potential for association with time to achieve the definition of improvement were then considered in a multivariable model.

For univariate analyses the association of the individual variables with time to achieve the definition of improvement was assessed using nonparametric comparisons of Kaplan-Meier curves (definition of improvement–free survival curves). Multicategory variables were grouped according to the quartiles of the observed values and evaluated using tests for trend. Nominal variables were assessed using Wilcoxon's homogeneity tests or log rank tests, and in the case of a substantial difference between the results of these two methods for a particular variable, that variable was entered into multivariate analysis if the P value was less than or equal to 0.01 by either method. The tests were performed using PROC LIFETEST in SAS version 9.3 (SAS Institute).

Results of the univariate analysis were illustrated with a hazard ratio (HR) ("hazard" of achieving the definition of improvement) for factors dichotomized at the median. HRs for nominal variables were computed with respect to the selected reference category. If the dichotomized variable had a similar

Table 2. Baseline values of univariate variables and their association with time to improvement*

Variable	Myositis patients (n = 195)	Category assessed	HR†	*P*‡
Demographic characteristics				
Age at diagnosis, years	40 (19–50)	≤40	1.12	0.11
Age at trial entry, years	46 (27–55)	≤46	1.06	0.16
Disease duration, years	3.3 (1.56–6.76)	≤3.3	1.34	0.06
Sex, no. (%) male	53 (27.2)	Male	1.27	0.10
Race, no. (%) white	138 (70.8)	White	1.28	0.22
Disease subset, no. (%) juvenile	48 (24.6)	Juvenile	1.15	0.06
Core set measures				
Manual Muscle Testing	74.6 (65–80)	≤74.6	1.11	0.52
Physician's global assessment of disease activity (100-mm VAS)	51 (38–62)	>51	1.08	0.50
Patient's/parent's global assessment of disease activity (100-mm VAS)	70 (51–82.6)	>70	1.02	0.66
HAQ/C-HAQ disability index	1.5 (1–2.13)	>1.5	1.09	0.08
Muscle enzyme, times the ULN	2.31 (1.17–7.35)	>2.31	1.30	0.10
Extramuscular VAS (100 mm)	25 (13.68–45.0)	>25	1.26	0.07
Autoantibody groups, no. (%)				<0.01§
Antisynthetase	30 (15.4)	Positive	2.83	<0.01
Anti–Mi-2	26 (13.3)	Positive	2.48	<0.01
Other autoantibodies	101 (51.8)	Positive	1.39	0.14
No autoantibodies	38 (19.5)	–	1.0 (reference)	
Medication				
Prednisone dosage, mean (25th–75th percentile)	21 (10–25)	>20	1.07	0.40
Number of failed immunosuppressive agents	3 (2–4)	≤3	1.08	0.35
Disease activity (100-mm VAS from MDAAT)				
Muscle disease activity	49.5 (30–63)	>49.5	1.27	0.85
Skeletal disease activity	0 (0–13)	>0	1.03	0.92
GI disease activity	0 (0–9)	>0	1.17	0.80
Pulmonary disease activity	3 (0–19)	>3	1.13	0.30
Disease damage (100-mm VAS from MDI)				
Muscle damage	23 (4–53)	≤23	1.26	<0.01
GI damage	0 (0–5.38)	0	1.03	0.27
Pulmonary damage	0 (0–9)	0	1.23	0.64
Physician's global assessment of damage	23 (10–45)	≤23	1.30	<0.01
Other clinical variables, no. (%)				
Mechanic's hands	35 (17.9)	Present	1.20	0.70
Dysphagia	152 (77.9)	Absent	1.10	0.44
Arthritis	16 (8.2)	Present	1.38	0.22
Active ILD¶	34 (20.1)	Present	1.12	0.99
Calcinosis#	32 (16.5)	Present	1.33	0.26
Raynaud's phenomenon	37 (19)	Present	1.14	0.57
Muscle atrophy**	70 (36.3)	Absent	1.45	0.02
Pulmonary fibrosis††	33 (18.6)	Present	1.38	0.11
Diminished lung function	30 (15.4)	Present	1.51	0.06
Laboratory variables				
Hemoglobin, gm/dl	39.9 (37.3–42.8)	≤39.9	1.13	0.97
Leukocyte (WBC) count, 10^9/liter	8.3 (6.6–11)	>8.3	1.44	0.06
Platelet count, 10^9/liter	296 (248–363)	>296	1	0.59
Total IgG, mg/dl	1,130 (890–1,470)	<1,130	1.02	0.67
Total IgM, mg/dl	112 (73–175)	<112	1.04	0.82
Serum creatinine, mg/dl	0.6 (0.4–0.7)	>0.6	1.12	0.57

* Except where indicated otherwise, values are the median (25th–75th percentile). VAS = visual analog scale; HAQ = Health Assessment Questionnaire; C-HAQ = Childhood HAQ; ULN = upper limit of normal; MDAAT = Myositis Disease Activity Assessment Tool; MDI = Myositis Damage Index; GI = gastrointestinal; ILD = interstitial lung disease; WBC = white blood cell.
† Hazard ratio (HR) for achieving the definition of improvement.
‡ By Wilcoxon's test (PROC LIFETEST in SAS version 9.3) or log rank test. The log rank test was used if the result was significantly different from that obtained with Wilcoxon's test.
§ *P* for difference among the 4 autoantibody groups.
¶ Data were available for 169 patients.
# Data were available for 194 patients.
** Data were available for 193 patients.
†† Data were available for 177 patients.

strength of association as its 4-category representation (results not shown), the binary form of the variable was considered in subsequent model building. In addition, all variables were analyzed separately in each arm of the trial to verify the absence of a masking effect of the treatment.

For the multivariable model, univariate factors with a P value of ≤ 0.1 were combined using a Cox proportional hazards model (PROC PHREG in SAS version 9.3). Variables in which a univariate association with definition of improvement was time-dependent were evaluated as time-dependent variables. Within the model, individual factors were tested at the 0.05 significance level. For each factor included in the final model, the HRs were evaluated at several time points. The 95% confidence intervals were adjusted for multiplicity using Scheffe's approach (PROC PHREG in SAS version 9.3).

In the secondary analyses to assess the influence of treatment on predictive factors, we analyzed the effect of treatment in the final multivariable model using treatment arm in both time-dependent and fixed variable approaches.

RESULTS

Patient characteristics. Data for a total of 195 of the 200 patients in the RIM trial (75 patients with PM, 72 patients with DM, and 48 patients with juvenile DM; 93 in the early treatment arm and 102 in the late treatment arm) were included in univariate analyses of baseline clinical, laboratory, autoantibody, and disease variables as predictors of clinical improvement. As previously reported (14), there was no difference in clinical outcomes between the rituximab early and late treatment groups. Most patients were Caucasian (70%) and female (73%), with a mean disease duration exceeding 5 years. The RIM trial population clearly had therapy-refractory disease and had features of active myositis. That is, the disease had already failed to respond to a mean of 3.1 immunosuppressive agents in these patients, but their mean baseline MDAAT physician's global assessment of disease activity and muscle activity VAS scores were 5.0 cm and 4.8 cm, respectively. The average prednisone dosage at study entry was 21 mg/day.

Eighty percent of the cohort (157 of 195) possessed at least one autoantibody as determined by IP (22). This included 30 patients (15%) with antisynthetases (28 with anti–Jo-1, 1 with anti-OJ, and 1 with anti–PL-7), 26 (13%) with anti–Mi-2, 25 (13%) with anti-SRP, 23 (12%) with anti–TIF-1γ, and 22 (11%) with anti-MJ. Twenty-four patients had other autoantibodies (e.g., anti-SSA/SSB, anti–U1 RNP, anti-U1/U2, anti-SAE, anti-Ku, or anticentromere), 9 had autoantibodies that were present but not clearly defined, and 38 patients had no identifiable autoantibodies. Of the 76 patients with DM enrolled at the beginning of the study, 16 (21%) had antisynthetases, 20 (26%) had anti–Mi-2, 30 (39%) had other autoantibodies, and 10 (13%) had no autoantibodies. Of the 76 patients with PM enrolled at the beginning of the study, 15 (20%) had antisynthetases, 1 (1%) had anti–Mi-2, 39 (51%) had other autoantibodies, and 21 (28%) had no autoantibodies. The subgroup of patients with juvenile DM included only 1 patient (2%) with antisynthetases, 5 patients (10%) with anti–Mi-2, 35 patients (73%) with other autoantibodies, and 7 patients (15%) without autoantibodies.

Univariate analyses of baseline patient characteristics that predicted time to improvement. The results of the univariate analyses of 38 predictor variables are summarized in Table 2. Twelve variables were identified as primary candidates for inclusion in the multivariable model ($P \leq 0.1$). Due to a similar definition of improvement-free survival (i.e., time to achieve the definition of improvement) for adult PM and DM RIM trial patients, and a difference in the juvenile DM subset, the myositis clinical subgroups were represented using a dichotomous factor differentiating adult myositis (PM and DM) from juvenile DM. The subgroup of patients with juvenile DM demonstrated a trend toward better clinical improvement as compared to the subgroups of adult patients ($P = 0.06$). Of the 12 primary candidates for the model, the following 4 had a significant ($P < 0.05$) univariate association with improvement: autoantibody group ($P = 0.001$), muscle damage ($P < 0.01$), physician's global assessment of damage ($P = 0.003$), and muscle atrophy ($P = 0.016$). Male sex, shorter disease duration, diminished lung function, higher leukocyte count, higher baseline muscle enzyme levels, higher extramuscular disease activity, and a higher HAQ/C-HAQ disability index univariately demonstrated a trend for association with improvement, but were not significant after accounting for other factors in the multivariable analysis. No additional factors were identified by considering each treatment arm separately.

The presence of a myositis autoantibody was most strongly associated with improvement and had a relatively constant effect on the time to achieve the definition of improvement throughout the trial. Specifically, the presence of an antisynthetase (primarily anti–Jo-1) and anti–Mi-2 were strongly related to achieving improvement (2–3 fold higher chances for improvement than for the subset with no autoantibodies; $P < 0.002$), while the "no autoantibody" group was associated with the worst time to improvement (Figure 1). Patients with other autoantibodies were more likely to improve (HR 1.4), although they were not significantly different from the group with no autoantibodies ($P = 0.14$).

Belonging to the clinical subgroup of juvenile

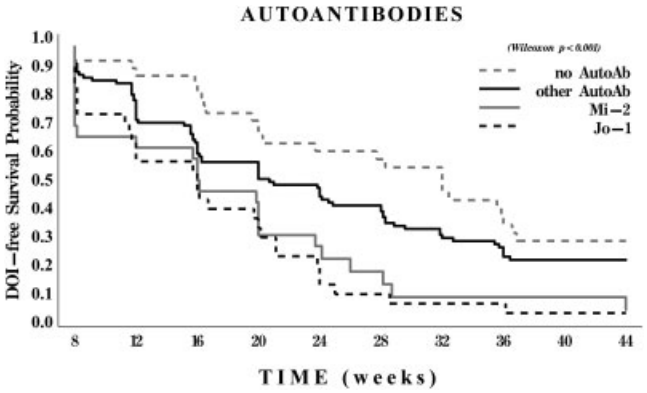

Figure 1. Kaplan-Meier curves for probability of meeting the definition of improvement (DOI) according to myositis autoantibody (autoAb) subset. Patients were classified into 4 subsets: those with antisynthetase autoantibodies (including anti–Jo-1), those with anti–Mi-2, those with other autoantibodies, and those with no detectable autoantibodies.

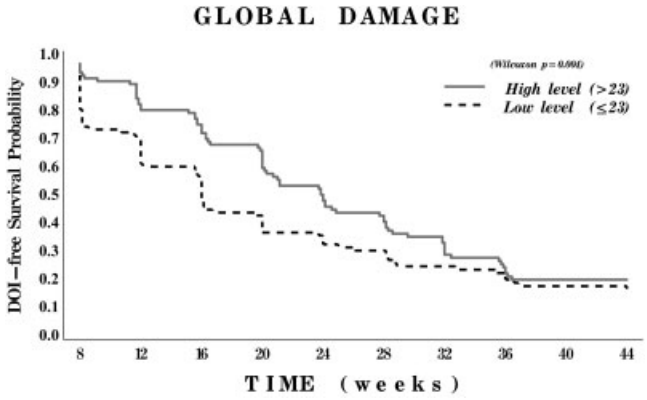

Figure 3. Kaplan-Meier curves for probability of meeting the definition of improvement (DOI) according to degree of myositis disease damage. Patients were classified as having low damage (score of ≤23 on a 100-mm visual analog scale [VAS]) or high damage (score of >23 on a 100-mm VAS).

DM (as compared to adult PM and DM) and lower physician's global assessment of damage were strong univariate predictors of time to improvement, but these effects decreased with time (Figures 2 and 3). There was no significant difference in time to improvement between the adult PM and adult DM clinical subgroups.

Multivariable analysis of baseline patient characteristics that predicted time to improvement. The final multivariable model included the following 3 significant factors associated with clinical improvement: autoantibody status (antisynthetase, anti–Mi-2, other autoantibodies, or no autoantibodies), physician's global assessment of damage (high [>23] or low [≤23] on a 100-mm VAS scale dichotomized at the median), and myositis subtype (adult or juvenile). The HRs for these 3 factors at weeks 8 and 20 are summarized in Table 3. Similar to the univariate assessment, after controlling for other factors in the multivariable model, patients with an antisynthetase (primarily anti–Jo-1) and those with anti–Mi-2 showed a 2–3-fold higher chance of improvement as compared to the "no autoantibodies" group. Lower physician's global assessment of damage and juvenile DM were associated with improvement in the final model. The time-varying nature of the effects of physician's global assessment of damage and myositis clinical subgroup were analyzed using interactions with time (as illustrated in Table 3). Over the first 8 weeks of the RIM trial, the time to improvement differed based on physi-

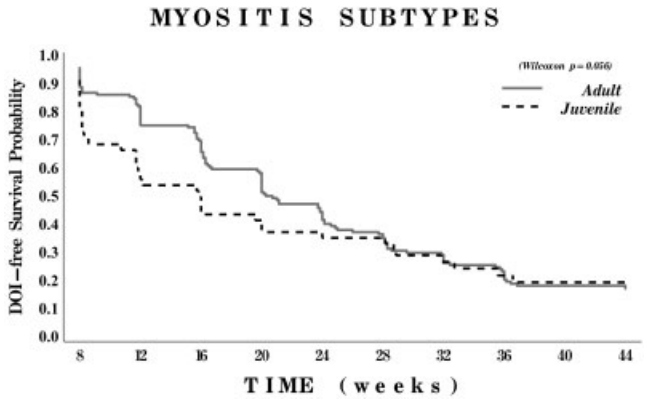

Figure 2. Kaplan-Meier curves for probability of meeting the definition of improvement (DOI) according to myositis clinical subgroup. Patients were classified as having either adult myositis (polymyositis or dermatomyositis) or juvenile dermatomyositis.

Table 3. Final multivariable model for predicting improvement*

Predictor variable	HR (95% CI)†	*P*
Autoantibody status		
No autoantibodies	1.0 (reference)	
Antisynthetase	3.08 (1.80–5.28)	<0.01
Anti–Mi-2	2.5 (1.42–4.41)	<0.01
Other autoantibodies	1.40 (0.90–2.17)	0.14
Physician's global assessment of damage (low vs. high)		
Week 8‡	2.32 (1.09–4.90)	0.02
Week 20‡	1.03 (0.66–1.60)	0.99
Myositis clinical subgroup (juvenile DM vs. adult PM/DM)		
Week 8‡	2.45 (1.16–5.15)	0.01
Week 20‡	1.01 (0.59–1.73)	0.41

* 95% CI = 95% confidence interval; DM = dermatomyositis; PM = polymyositis.
† Hazard ratio (HR) of achieving the definition of improvement.
‡ Values are adjusted for multiplicity using Scheffe's method (PROC PHREG in SAS version 9.3).

cian's global assessment of damage scores and adult PM/DM versus juvenile DM subgroups. However, after week 20 of the trial, there were no significant differences in improvement between these groups. In contrast, the presence of autoantibodies was associated with substantial differences in improvement throughout the entire duration of the RIM trial.

The time-dependent effect of treatment (i.e., the 8-week lag for the late treatment arm) was not associated with improvement, and the results of multivariable analysis were not affected after controlling for treatment arm.

DISCUSSION

Of the patients enrolled in the RIM trial, 83% experienced improvement (met the definition of improvement), while historically 30% of patients with refractory myositis improve (23,24). The present study indicates that the presence of autoantibodies, especially the antisynthetases (mainly anti–Jo-1) and anti–Mi-2, was the strongest predictor of clinical improvement in a cohort of rituximab-treated myositis patients, whereas a lack of definable autoantibodies predicted no improvement. In patients in all other autoantibody subgroups, including those with anti-SRP, the disease had similar responses, with similar predictive capacity of different autoantibodies. We found no differences between anti–Jo-1 and anti–Mi-2 as predictors of improvement, although both were predictive of a shorter time to improvement than anti-SRP or other autoantibodies. Previous reports indicated that anti–Jo-1–positive patients required more immunosuppressive medication than patients with anti–Mi-2, although both responded better than anti-SRP–positive patients (6,7,25). This is somewhat consistent with our results, which showed the superior rate and time to improvement of anti–Jo-1–positive and anti–Mi-2 positive patients.

We found that patients with juvenile DM and patients with a lower degree of myositis damage at baseline were more likely to have a favorable clinical improvement in a cohort of rituximab-treated myositis patients. In the RIM trial there was no difference in response rates between the early and late treatment arms. In this analysis we did not detect a treatment effect of early or late treatment after accounting for significant predictors of improvement.

The importance of myositis autoantibodies to identify phenotypically distinct subsets of myositis patients is well recognized, and our results expand their role as predictive factors for clinical improvement in myositis patients. Our finding that anti–Jo-1 predicts clinical improvement is even more intriguing when considered in the context of previous studies demonstrating that anti–Jo-1 autoantibody levels may serve as a biomarker of myositis disease activity (26–28). Combined with additional RIM trial data showing that anti–Jo-1 autoantibody levels correlate with myositis disease activity (29), the findings reported herein that Jo-1–positive patients have a better outcome suggests that immune responses related to anti–Jo-1 autoantibodies may be pathogenic. In this regard, human tyrosyl–transfer RNA synthetase, a rare autoantigen in myositis, has chemoattractant and leukocyte-activating properties after proteolytic cleavage (30, 31), while histidyl–transfer RNA synthetase (the target of anti–Jo-1) and asparaginyl–transfer RNA synthetase (the target of anti-KS) activate chemokine receptors on T lymphocytes and immature dendritic cells (32). Thus, autoantibodies directed against these ubiquitous human aminoacyl–transfer RNA synthetases or the antigens themselves may contribute in some undetermined manner to the perpetuation of pathogenic immune responses in muscle or other tissue (32).

Similarly, our results demonstrated that patients with anti–Mi-2 also had better clinical improvement. Previous studies have shown anti–Mi-2 to be associated with more favorable outcomes (33,34). In contrast, patients with anti-SRP generally have necrotizing, poorly responsive PM (6,35), which is similar to our finding of intermediate improvement of patients with anti-SRP autoantibodies (worse than those with anti–Jo-1 and anti–Mi-2) in the RIM trial. In a separate study analyzing comparative survival among patients stratified by autoantibody, we recently showed similar survival among patients with anti-SRP, those with anti–Jo-1, and those with anti–Mi-2 (36). However, survival may be very different from clinical improvement in a therapeutic trial. In contrast to autoantibody-positive patients, those with no definable myositis autoantibodies had a worse outcome, suggesting that possessing an autoantibody may predict a favorable prognosis, even in autoantibody subsets known to have a worse prognosis (i.e., anti-SRP). Anti-SRP autoantibody–positive patients showed similar improvement to patients with "other" autoantibodies, but worse than those with anti–Jo-1 and those with anti–Mi-2, and fractionally better than groups with no myositis autoantibodies. Although we grouped all antisynthetase autoantibodies together, the predominant autoantibody was anti–Jo-1; thus, these results cannot be applied to non–Jo-1 autoantibodies (e.g. anti–PL-7, anti–PL-12, etc.). In fact, recent evidence

suggests that patients with non–Jo-1 autoantibodies have worse survival than patients with Jo-1 antibody (36).

Anti–melanoma differentiation–associated protein 5 and anti–hydroxymethylglutaryl-coenzyme A are 2 newly characterized myositis autoantibodies that were not measured in the RIM trail, and patients who were positive for these autoantibodies might have been included in the "no autoantibody" group. Many PM patients were autoantibody negative, and there may be a concern that some of these patients represent PM mimics (e.g., patients with IBM or adult muscular dystrophy), leading to a poor outcome in this group (37). However, a 3-member adjudication committee of myositis experts including a neurologist/neuropathologist reviewed the medical records and muscle biopsy findings to insure that PM mimics were excluded from the RIM trial. Despite these limitations, the present study is the first to comprehensively demonstrate that autoantibodies are major predictive factors of clinical improvement in myositis patients treated with B cell–depleting therapy.

The present study also showed that lower global damage predicts clinical improvement. Previously published studies have shown that muscle damage in myositis is a marker of a poor prognosis (10). All 3 measures of damage (muscle damage and atrophy and physician's global assessment of damage) were strongly associated with poor clinical improvement in univariate analyses. Physician's global assessment of damage had the strongest association with improvement and was the only damage variable that remained in the final multivariable model, since muscle damage and atrophy did not substantially add to the prediction of improvement. Importantly, lower damage predicted a favorable outcome early in the course of the study, as the association with improvement decreased after week 20. The reasons for this are not clear, although it would seem plausible to postulate that patients with more damage at baseline demonstrate a delayed improvement.

This is the first study to directly demonstrate a better outcome in patients with juvenile DM than in those with adult myositis. A Korean trial showed superior survival and clinical outcomes in juvenile DM patients compared to adult DM patients (38), while other studies have demonstrated better long-term survival of patients with juvenile DM (20,39). An older age at onset has been recognized as a marker of poor prognosis with regard to survival in myositis patients (19,20,40), and younger patients have higher remission rates (41). However, we specifically demonstrated that juvenile DM subgroup, and not age at diagnosis, predicted a favorable and more rapid clinical improvement in the final model. Also, these results in juvenile DM patients are not attributable to a shorter disease duration. Similar to the observation with lower damage, juvenile DM predicted a favorable outcome early in the course of the study, as the association with improvement decreased after week 20.

Juvenile DM patients have been shown to have lower myositis-related damage as compared to adult PM/DM patients (42); however, as we demonstrated, the chances of improvement were much higher for juvenile DM patients even after adjusting for global damage. Juvenile DM was associated with substantial improvement early in the trial compared to either adult DM or adult PM; however, these individual differences were not statistically significant for the available sample sizes. The adult DM and PM subsets had similar definition of improvement–free experiences and were therefore combined for all analyses. The difference in definition of improvement–free survival between the juvenile DM group and the combined group of adult PM and DM patients was similar to the difference between the juvenile DM group and the adult DM group alone and the difference between the juvenile DM group and the adult PM group alone.

It is important to recognize that in our study, univariate and multivariable analyses were used to predict the association with clinical improvement in adult and juvenile myositis. This should not be interpreted as an overall predictor of response to rituximab, since all patients received rituximab in the RIM trial. Thus, it is difficult to conclude whether the predictive factors identified are valid for myositis patients in general or only those treated with B cell depletion. Nevertheless, given that rituximab is a B cell–depleting agent that may directly inhibit autoantibody production, it is plausible that the favorable results in myositis autoantibody–producing patients as compared to those without autoantibodies are at least partly due to rituximab.

In summary, we found certain autoantibodies to be the strongest predictive markers of clinical improvement in a cohort of rituximab-treated myositis patients. Patients with antisynthetase autoantibodies (predominantly anti–Jo-1) and anti–Mi-2 had a better outcome, and the absence of myositis autoantibodies was associated with a worse outcome. We also found that myositis disease-associated damage, and specifically muscle damage, were markers of a poor clinical outcome, while juvenile DM predicted a better outcome as compared to adult DM or PM. However, low myositis damage and juvenile DM were associated with more rapid improve-

ment only early in the course of the study. We believe these findings improve our understanding of the pathogenesis of myositis and provide important guidelines for the design of future IIM clinical trials. Perhaps future clinical trials in myositis should be designed to analyze juvenile DM and adult myositis groups separately, stratify patients based on level of global damage, and account for autoantibodies in the analysis.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Aggarwal had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Aggarwal, Reed, Ascherman, Feldman, Miller, Rider, Levesque, Oddis.

Acquisition of data. Aggarwal, Reed, Ascherman, Barohn, Feldman, Miller, Rider, Harris-Love, Oddis.

Analysis and interpretation of data. Aggarwal, Bandos, Reed, Ascherman, Miller, Oddis.

ROLE OF THE STUDY SPONSOR

Genentech had no role in the study design or in the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to submit the manuscript for publication. Publication of this article was not contingent upon approval by Genentech.

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APPENDIX A: RIM STUDY GROUP MEMBERS

Members of the RIM Study Group (countries, principal investigators, and centers) are as follows: in Canada (pediatric sites), Brian Feldman (Hospital for Sick Children, Toronto, Ontario) and Adam Huber (IWK Health Centre, Halifax, Nova Scotia); in the Czech Republic (adult site), Jiří Vencovský and Herman Mann (Institute of Rheumatology, Prague); in Sweden (adult site), Ingrid E. Lundberg (Karolinska Institutet, Stockholm); in the US (adult sites), Richard Barohn, Mazen Dimachkie, and Kevin Latinis (University of Kansas Medical Center, Kansas City), Lorinda Chung and David Fiorentino (Stanford University, Palo Alto, CA), Leslie Crofford (University of Kentucky, Lexington), Mary Cronin (Medical College of Wisconsin, Milwaukee), Stephen DiMartino (Hospital for Special Surgery, New York, NY), Barri Fessler (University of Alabama at Birmingham), Michael Harris-Love (Washington DC VA Medical Center), Sharon Kolasinski (University of Pennsylvania, Philadelphia), Todd Levine (Phoenix Neurological Associates, Phoenix, AZ), Galina Marder (North Shore–LIJ, New York, NY), Richard Martin and Aaron Eggebeen (Michigan State University, Grand Rapids [adult and pediatric site]), Frederick Miller (National Institute of Environmental Health Sciences, NIH, Bethesda, MD), Pushpa Narayanaswami and Seward B. Rutkove (Beth Israel Deaconess Medical Center/Harvard Medical School, New York, NY), Chester Oddis, Dana Ascherman, Rohit Aggarwal, David Lacomis, and Christopher Bise (University of Pittsburgh, Pittsburgh, PA), Nancy Olsen and Andreas Reimold (University of Texas Southwestern Medical Center at Dallas), Elena Schiopu, Kristine Phillips, and James Seibold (University of Michigan, Ann Arbor), Khema Sharma (University of Miami, Miami, FL), Swamy Venturupalli and Michael Weisman (Cedars-Sinai Medical Center, University of California at Los Angeles), and Steven Ytterberg (Mayo Clinic, Rochester, MN); in the US (pediatric sites), Susan Kim (Children's Hospital of Boston, Boston, MA), Tzielan Lee (Stanford University, Palo Alto, CA), Daniel Lovell (Cincinnati Children's Hospital, Cincinnati, OH), C. Egla Rabinovich (Duke University Medical Center, Durham, NC), Ann Reed (Mayo Clinic, Rochester, MN), Lisa Rider (National Institute of Environmental Health Sciences, NIH, Bethesda, MD), Rafael Rivas-Chacon (Miami Children's Hospital, Miami, FL), and David Sherry (The Children's Hospital of Philadelphia, Philadelphia, PA).