Minimal residual disease flow cytometry of peripheral blood B-cell subsets in patients with idiopathic inflammatory myopathies following rituximab therapy: a single-centre, retrospective cohort study

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Summary

Background Rituximab treatment has shown inconclusive results in clinical trials and observational studies in patients with idiopathic inflammatory myopathies. This study aimed to assess whether complete depletion of distinct B-cell subsets was associated with clinical response in rituximab-treated patients with myositis using highly sensitive flow cytometry (HSFC).

Methods This single-centre, retrospective cohort study included adults (aged ≥18 years) treated with rituximab who fulfilled the American College of Rheumatology (ACR) and European Alliance of Associations for Rheumatology (EULAR) classification criteria for idiopathic inflammatory myopathies, followed up at the Leeds Teaching Hospitals NHS Trust (Leeds, UK). HSFC was performed before the first rituximab infusion and at 2 weeks. The primary outcome was a clinical response (at least moderate improvement on the 2016 ACR–EULAR total improvement score) at the first follow-up 4–6 months after infusion. Associations of clinical response with complete HSFC depletion (<0·1 cells per μL) of naive B cells, memory B cells, and plasmablasts versus the conventional flow cytometry cutoff (<5 total B cells per μL) were assessed using multivariable Firth penalised logistic regression. No patients with lived experience were involved in the study.

Findings Between Jan 1, 2015, and Dec 31, 2025, 44 patients with idiopathic inflammatory myopathies were treated with rituximab and had complete datasets. Of these patients, 24 (55%) had dermatomyositis, 12 (27%) had antisynthetase syndrome, five (11%) had immune-mediated necrotising myositis, and three (7%) had polymyositis. 17 (39%) of 44 patients were male, 27 (61%) were female, 35 (80%) were White, and the median age was 45 years (IQR 38–56). Rituximab response occurred in 31 (70%) patients. The total improvement score was higher in responders than non-responders (median 50·0 [IQR 45·0–55·0] *vs* 17·5 [10·0–30·0]; p<0·0001). At 2 weeks, memory B-cell counts and plasmablast counts were lower in responders than non-responders and complete plasmablast depletion was significantly more frequent in responders (29 [94%] of 31 *vs* four [31%] of 13; p<0·0001). In multivariable models, higher 2-week plasmablast counts were associated with lower odds of response (odds ratio 0·03 [95% CI 0·00–0·23]), whereas depletion of memory B cells (8·68 [1·13–82·80]) and plasmablasts (25·20 [4·61–214·54]) was associated with response. The conventional flow cytometry cutoff showed no association with response.

Interpretation Complete early B-cell depletion, measured using an early HSFC-based strategy, but not conventional flow cytometry, was associated with rituximab response in patients with idiopathic inflammatory myopathies, supporting further evaluation of a peripheral blood B-cell minimal residual disease framework as a pharmacodynamic and prognostic strategy. These findings could help to select and optimise advanced B-cell depleting strategies in patients with refractory inflammatory myopathies.

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Introduction

B lymphocytes are implicated in the pathogenesis of idiopathic inflammatory myopathies,[1] in which distinct subsets—including naive B cells and plasmablasts—are enriched compared with the same subsets in healthy individuals.[2–4] This B-cell involvement is mirrored by decades of work on myositis-specific and myositis-associated autoantibodies,[5,6] which has driven a critical revision of the original phenotype-based classification of idiopathic inflammatory myopathies towards an autoantibody-based framework with clinical correlates, including associations with visceral involvement and cancer.[7–9]

Despite this progress, idiopathic inflammatory myopathies remain orphan diseases. B-cell depleting approaches, mostly rituximab, have been variably

Research in context

Evidence before this study

We searched PubMed from database inception to March 31, 2026, for studies on rituximab and B-cell depletion in patients with idiopathic inflammatory myopathies. Search terms included "myositis", "dermatomyositis", "polymyositis", "antisynthetase", "rituximab", "B cells", "plasmablasts", and "flow cytometry". Highly sensitive flow cytometry (HSFC)-defined minimal residual disease is associated with response to rituximab in haematological malignancies and autoimmune diseases (rheumatoid arthritis and systemic lupus erythematosus), where incomplete early depletion of memory B cells and plasmablasts is associated with subsequent treatment non-response. This paradigm has not been investigated in patients with myositis, in whom rituximab shows variable efficacy with heterogeneous responses and frequent relapse. No validated early biomarker has been found to predict clinical outcomes.

Added value of this study

This retrospective cohort study provides preliminary evidence that an HSFC-defined minimal residual disease framework might be clinically informative in patients with idiopathic inflammatory myopathies treated with rituximab. In one of the largest, deeply immunophenotyped cohorts with detailed B-cell subset analysis to date, we show that the depth of B-cell depletion—rather than its binary achievement using conventional thresholds—is crucial. Complete, early depletion of memory B cells and plasmablasts on HSFC (<0·1 cells per µL at 2 weeks) was associated with subsequent clinical response, whereas their persistence was associated with non-response. Conventional flow cytometry cutoffs (<5 cells per µL) did not capture these associations. In initial responders, re-emergence of minimal residual disease-detectable B cells showed an exploratory association with subsequent relapse. These findings extend a biologically grounded minimal residual disease paradigm—well established in haematology—into idiopathic inflammatory myopathies, providing a quantifiable biomarker linking early pharmacodynamic effects to subsequent clinical outcomes.

Implications of all the available evidence

These findings suggest that heterogeneity in rituximab efficacy in patients with idiopathic inflammatory myopathies might reflect variability in achieving deep depletion of pathogenic B-cell lineages. HSFC-based minimal residual disease monitoring could enable early stratification of patients into adequate versus inadequate responders, supporting timely treatment optimisation. In the context of emerging potent therapies targeting B cells and plasma cells (eg, type II anti-CD20 antibodies, bispecific T-cell engagers, and CAR T cells), this framework could contribute to a clinically actionable strategy to guide escalation decisions and personalise treatment pathways. Adoption of minimal residual disease-based endpoints could refine trial design and accelerate precision medicine in myositis. Prospective studies should determine whether minimal residual disease-inspired measures could be used as trial endpoints.

adopted, with some evidence of efficacy but highly heterogeneous response rates, and relapse after initial response.[10] Few reports using more advanced strategies—such as chimeric antigen receptor (CAR) T cells targeting $CD19^+$ B cells as well as B-cell maturation antigen $(BCMA)^+$ plasma cells—suggest that deep B lineage depletion could be effective in patients with idiopathic inflammatory myopathies.[10–13] Although rituximab is widely used for different myositis manifestations, including muscle, skin, and lung involvement,[14] there is no validated mechanistic biomarker that predicts clinical response[15] or risk of subsequent relapse. As rheumatology moves towards advanced B-cell depleting strategies, assessing B-cell lineages in association with rituximab response could help inform target treatment.

Highly sensitive flow cytometry (HSFC) is used in haematology settings to quantify low concentrations of circulating B cells after depleting therapies (ie, minimal residual disease).[16] This least detectable pathological cell population is a major predictor of disease remission, recurrence, or progression in haematological neoplasms (eg, B-cell and plasma cell lymphoproliferative diseases).[16,17] Compared with conventional flow cytometry (5 cells per µL), HSFC is characterised by a 2-log more sensitive limit of detection for B cells (0·1 cells per µL) and the ability to discriminate different B-cell subsets: naive, memory B cells, and plasmablasts.[16,18] Plasmablasts are often lost during sample preparation for conventional techniques. By using HSFC, but not conventional flow cytometry, distinct residual B-cell subsets (memory B cells and plasmablasts) 2 weeks after the first rituximab administration were shown to be associated with impaired later clinical response in patients with rheumatoid arthritis, whereas their complete depletion was associated with response.[18,19] Likewise, HSFC predicted rituximab responses in patients with systemic lupus erythematosus (SLE) and antineutrophil cytoplasmic antibody-associated vasculitis.[18–21] In a randomised, placebo-phase trial by Oddis and colleagues, rituximab did not meet its primary endpoint, thus questioning the validity of B-cell depletion in patients with idiopathic inflammatory myopathies; however, some heterogeneity in responses was noted, underscoring the need to identify patients most likely to respond (including an evaluation of B-cell biomarkers).[10,11]

To date, HSFC has not been used in patients with idiopathic inflammatory myopathies treated with rituximab. However, as B-cell targeting and plasma cell

targeting strategies are rapidly expanding, data linking highly sensitive B-cell enumeration with rituximab responsiveness could have important clinical implications, including selecting therapeutic escalation to more potent monoclonal antibodies, CAR T cells, or bispecific T-cell engagers in patients with detectable minimal residual disease (here intended as the persistence of residual circulating B-cell subsets despite treatment).

This study aimed to assess whether HSFC-defined complete B-cell depletion—corresponding to an undetectable circulatory minimal residual disease state—was associated with subsequent clinical response in patients with myositis, compared with conventional flow cytometry cutoffs. Finally, we assessed the association between repopulation of B-cell subsets (ie, re-emergence of detectable minimal residual disease) and disease relapse.

Methods

Study design and participants

This single-centre, retrospective cohort study included adults (aged ≥18 years) fulfilling the American College of Rheumatology (ACR)–European Alliance of Associations for Rheumatology (EULAR) classification criteria for idiopathic inflammatory myopathies,[22] who were followed up at the rheumatology service of the Leeds Teaching Hospitals National Health Service (NHS) Trust (Leeds, UK) and started rituximab treatment (two intravenous infusions of 1 g 2 weeks apart). All patients had complete clinical and HSFC data available at the first rituximab infusion (baseline) and at 2 weeks. For every patient, a follow-up visit documenting clinical response was available 4–6 months after the first rituximab infusion. In a subset of patients who responded to the first rituximab cycle and for whom further follow-up was available, HSFC data were additionally collected after at least 6 months from the documented response, as part of routine B-cell monitoring in patients treated with rituximab.

People with lived experience were not involved in study design or conduct. Informed consent and formal ethics approval was not required because this was a retrospective study conducted as part of a service evaluation, and all treatment decisions were made before the evaluation of data, in accordance with NHS Research Ethics Committee guidelines.

Procedures

Collected variables included participant demographics (age, sex [retrieved from electronic health records], ethnicity [retrieved from electronic health records], and disease duration at the time of the first rituximab infusion) and disease domains including heliotrope rash, Gottron's sign or papules, Raynaud's phenomenon, mechanic's hands, alopecia, interstitial lung disease on high-resolution chest CT,[23] calcinosis, arthritis, dysphagia, and myopathy (clinical evidence of symmetrical and proximal muscle weakness judged by an experienced clinician plus at least one of the following: raised creatine kinase larger than two times above the local laboratory upper limit of normal, oedema on muscle MRI, or a muscle biopsy consistent with inflammation [or a combination]). Myositis-specific and myositis-associated autoantibodies were assessed using EUROLINE immunoblot (Euroimmun; London, UK), which allows the simultaneous detection of antibodies to Jo-1, PL-7, PL-12, EJ, OJ, MDA5, Mi-2, NXP2, SAE1, SRP, TIF1-γ, Ku, PM/Scl, and Ro52. Anti-HMGCR antibodies were either detected through dedicated ELISA (Inova Diagnostics; San Diego, CA, USA) or as part of the EUROLINE immunoblot, depending on kit availability when the test was done. Autoantibody detection was done in all patients at the time of myositis diagnosis, according to standard procedures and routine laboratory procedures. Cancer-associated myositis was defined as inflammatory myopathy onset within 3 years of a malignancy diagnosis.[8] Previous treatment history for myositis was also recorded. Idiopathic inflammatory myopathy phenotypes were classified as dermatomyositis, antisynthetase syndrome, polymyositis, and immune-mediated necrotising myositis, according to established definitions.[1,7]

Glucocorticoids and immunosuppressive treatments administered before and concomitant with rituximab were recorded. Of patients receiving glucocorticoids or immunosuppressants concomitant with rituximab, none received intensification of the underlying treatment (ie, dose increase or rescue bursts of glucocorticoids, initiation or dose escalation of conventional synthetic disease-modifying antirheumatic drugs, or immunomodulatory drugs) in the period between the first rituximab course and when response was assessed (ie, the first available visit 4–6 months after the first infusion), as per standard operating procedures.

Peripheral naive, memory B cells and plasmablasts were enumerated by HSFC as previously described[18] and are summarised in the appendix (p 1). Absolute B-cell counts were obtained using standard cell surface staining procedures and a sequential gating strategy with six-colour flow cytometry (CD3, CD14, CD19, CD27, CD38, and CD64), acquiring a minimum of 500 000 leucocyte events. Absolute B-cell subset counts were calculated using standard cell surface staining procedures. A sequential fluorescence-first gating strategy was applied, without an initial lymphocyte scatter gate, to prevent exclusion of plasmablasts, which have weak or absent CD19 expression, reduced CD45 expression, and light scatter characteristics extending outside the standard lymphoid region.[24] Contaminating events were excluded using separate dump channels for $CD3^+$ T cells and $CD14^+CD64^+$ monocytes. Naive B cells were identified as $CD19^+CD3^-CD14^-CD64^-CD27^-$ events, followed by application of a singlet lymphoid scatter gate as a final

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step. Memory B cells were identified as CD19⁺CD3⁻CD14⁻CD64⁻CD27⁺ events, with subsequent selection of CD19⁺CD38⁻ events to exclude plasmablasts, followed by a singlet lymphoid scatter gate. Plasmablasts were identified as CD3⁻CD14⁻CD64⁻CD27⁺ events, with subsequent selection of cells with a very high expression of CD38 and CD27 (CD19 expression being variable and potentially absent). A separate scatter gate encompassing the blast zone, distinct from the lymphocyte scatter gate, was applied as a final step to exclude doublets and noise. Complete depletion of each subset was defined as less than 0·1 cells per µL (HSFC sensitivity threshold), compared with the conventional flow cytometry threshold of less than 5 total B cells per µL.

	Whole cohort (n=44)	Responders (n=31)	Non-responders (n=13)	p value
Demographics				
Age at myositis onset, years	45 (38–56)	47 (40–56)	43 (33–55)	0·34
Follow-up duration, years	5 (3–10)	5 (3–10)	5 (3–10)	0·94
Sex	..	..	..	0·17
Female	27 (61%)	17 (55%)	10 (77%)	..
Male	17 (39%)	14 (45%)	3 (23%)	..
Ethnicity	..	..	..	0·44
White	35 (80%)	24 (77%)	11 (85%)	..
Asian	7 (16%)	6 (19%)	1 (8%)	..
African	1 (2%)	0	1 (8%)	..
Hispanic	1 (2%)	1 (3%)	0	..
Clinical symptoms				
Heliotrope rash	23 (52%)	18 (58%)	5 (38%)	0·24
Gottron's sign or papules	20 (45%)	12 (39%)	8 (62%)	0·17
Raynaud's phenomenon	14 (32%)	12 (39%)	2 (15%)	0·17
Mechanic's hands	11 (25%)	5 (16%)	6 (46%)	0·057
Alopecia	2 (5%)	1 (3%)	1 (8%)	0·51
Myopathy	30 (68%)	22 (71%)	8 (62%)	0·72
Interstitial lung disease	21 (48%)	15 (48%)	6 (46%)	0·89
Calcinosis	3 (7%)	2 (6%)	1 (8%)	1·00
Arthritis	17 (39%)	12 (39%)	5 (38%)	0·99
Oropharyngeal dysphagia	7 (16%)	6 (19%)	1 (8%)	0·65
Autoantibodies				
Anti-nuclear antibodies	32 (73%)	23 (74%)	9 (69%)	0·73
Anti-Ro52	13 (30%)	11 (35%)	2 (15%)	0·28
Anti-PM/Scl	6 (14%)	5 (16%)	1 (8%)	0·65
Antisynthetase*	12 (27%)	8 (26%)	4 (31%)	0·73
Anti-MDA5	7 (16%)	4 (13%)	3 (23%)	0·40
Anti-Mi-2	5 (11%)	4 (13%)	1 (8%)	1·00
Anti-TIF1-γ	3 (7%)	1 (3%)	2 (15%)	0·20
Anti-SRP	2 (5%)	0	2 (15%)	0·083
Anti-HMGCR	4 (9%)	3 (10%)	1 (8%)	1·00
Inflammatory myopathy subset				
Antisynthetase syndrome	12 (27%)	8 (26%)	4 (31%)	1·00
Dermatomyositis	24 (55%)	17 (55%)	7 (54%)	..
Necrotising myositis	5 (11%)	4 (13%)	1 (8%)	..
Polymyositis	3 (7%)	2 (6%)	1 (8%)	..
Disease activity at baseline				
Physician global assessment (0–10)	7·0 (7·0–8·0)	7·0 (7·0–8·0)	7·0 (7·0–8·0)	0·82
Patient global assessment (0–10)	7·5 (7·0–8·0)	7·5 (7·0–8·0)	7·5 (7·0–8·0)	0·61
Manual muscle testing (0–150)	144 (134–149)	144 (132 –150)	140 (136 –146)	0·50
Health assessment questionnaire (0–3)	1·0 (0·5–1·6)	0·9 (0·3–1·6)	1·1 (0·7–1·9)	0·26
Extramuscular disease activity (0–10)	7·0 (5·0–8·0)	6·0 (4·5–8·0)	7·0 (6·0–8·0)	0·35
Creatine kinase, units per L	130 (62–603)	118 (68 –515)	141 (56–690)	0·88
Serum C-reactive protein, mg/L	5·5 (0·0–10·0)	5·0 (0·0–11·0)	5·6 (2·5–9·0)	0·67

(Table 1 continues on next page)

Outcomes

Exposures of interest were absolute cell counts of naive B cells, memory B cells, and plasmablasts at baseline and 2 weeks after the first rituximab infusion and evidence of complete depletion of each subset on HSFC at 2 weeks post-infusion (<0·1 cells per µL [ie, undetectable minimal residual disease]). The conventional flow cytometry cutoff served as comparator (<5 total B cells per µL without distinguishing the three subpopulations).

Clinical response to rituximab (primary outcome) was defined as having moderate or major improvement according to the 2016 ACR–EULAR response criteria[25] and was assessed at the first available visit 4–6 months after the first infusion, in line with routine clinical practice. The 2016 ACR–EULAR criteria quantify the change across six core set measures: physician global disease activity (0–10), patient global disease activity (0–10), manual muscle testing score, Health Assessment Questionnaire score (0–3), extramuscular disease activity (0–10), and serum creatine kinase concentration (IU/L). The total improvement score (0–100) was calculated according to these criteria and categorised as major (≥60), moderate (≥40), minimal (≥20), or no improvement.[25] In this study, patients with moderate or major improvement were considered to have had a clinical response.

In patients who had a clinical response, subsequent relapse was analysed as a secondary, exploratory outcome. In the absence of a shared established definition, relapse was defined as clinically meaningful worsening, in keeping with the 2016 ACR–EULAR criteria, in the domains prompting rituximab initiation (myositis, skin involvement, lung involvement, and arthritis), occurring at least 6 months after having a clinical response and leading to treatment modification (switching or escalation of immunosuppressive therapy, retreatment with rituximab, or initiation or dose escalation of glucocorticoids). For the relapse analysis, the exposure of interest was B-cell subset repopulation, defined by the detection of more than 0·1 cells per µL (ie, detectable minimal residual disease) on routinely performed HSFC at least 6 months from the documented response. Relapse status was ascertained from clinical data recorded at the same (later HSFC) timepoint (±2 months).

Statistical analysis

Continuous variables were assessed for distribution using the Shapiro-Wilk test and are presented as mean

(SD) or median (IQR), as appropriate; groups were compared using the *t* test or Mann-Whitney test. For patients with available HSFC follow-up data, within-patient changes (delta) in absolute B-cell subset counts were calculated as the value at later timepoint minus the value at baseline and compared between groups using non-parametric tests, as appropriate. Categorical variables are reported as counts and percentages and were compared using the Chi squared test or Fisher's exact test, as appropriate. Spearman's correlation analysis was done to explore for correlations between baseline cell counts and disease activity measures.

Associations with the primary outcome were evaluated using multivariable logistic regression. Covariates included age, sex, and variables with a p value less than 0·10 in univariable analysis. Several prespecified models were fitted, including demographic covariates only, demographics plus the clinical variable mechanic's hands, and demographics plus anti-SRP autoantibodies. Log-transformed absolute B-cell counts were used in regression models according to the formula: log-transformed=log (count×10+1),[19] to reduce skewness and handle zero counts. Given the sample size, Firth's penalised likelihood approach was used to fit multivariable logistic regression models, and the number of predictors was constrained in relation to the number of outcome events to limit overfitting. Collinearity was assessed using variance inflation factor; models included either absolute B-cell counts or binary depletion variables to avoid collinearity. Complete-case analysis was performed: the number of missing variables was zero for the primary outcome, and only complete cases were analysed for the secondary outcome. Statistical significance was defined as a two-tailed p-value of less than 0·05. Analyses were performed in R (version 4.4.1).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Jan 1, 2015, and Dec 31, 2025, 44 patients with idiopathic inflammatory myopathies were treated with rituximab and had complete datasets. Of these, 24 (55%) had dermatomyositis, 12 (27%) had antisynthetase syndrome, five (11%) had immune-mediated necrotising myositis, and three (7%) had polymyositis; no patients had overlap myositis (table 1). 27 (61%) of 44 patients were female and 17 (39%) were male. 35 (80%) patients were White. Median age was 45 years (IQR 38–56), and the median follow-up time was 5 years (IQR 3–10). No patients had cancer-associated myositis. Clinical manifestations included myopathy in 30 (68%) patients, heliotrope rash in 23 (52%), Gottron's papules or sign in 20 (45%), interstitial lung disease in 21 (48%), arthritis in 17 (39%), Raynaud's phenomenon in 14 (32%), and oropharyngeal dysphagia in seven (16%). Five (11%) patients were negative for any myositis-specific or myositis-associated autoantibody, 14 (32%) patients had two autoantibodies detected (11 responders, three non-responders), and one patient (2%; non-responder) was simultaneously positive for anti-SRP, anti-Ro52, and anti-PM/Scl (table 1).

Previous immunosuppressive treatments included methotrexate in 16 (36%) patients, cyclophosphamide in 12 (27%), mycophenolate mofetil in eight (18%), azathioprine in three (7%), and ciclosporin A in two (5%). At the time of the first rituximab infusion, 23 (52%) of 44 patients were receiving at least 10 mg/day of glucocorticoids, 19 (43%) were receiving mycophenolate mofetil, five (11%) were receiving azathioprine, four (9%)

	Whole cohort (n=44)	Responders (n=31)	Non-responders (n=13)	p value
(Continued from previous page)				
Treatments at baseline				
Glucocorticoids	23 (52%)	14 (45%)	9 (69%)	0·14
Intravenous immunoglobulins	4 (9%)	3 (10%)	1 (8%)	1·00
Immunosuppressants	28 (64%)	19 (61%)	9 (69%)	0·74
Methotrexate	4 (9%)	2 (6%)	2 (15%)	0·60
Mycophenolate mofetil	19 (43%)	15 (48%)	4 (31%)	0·28
Azathioprine	5 (11%)	2 (6%)	3 (23%)	0·14
Previous treatments†				
Methotrexate	16 (36%)	12 (39%)	4 (31%)	0·74
Mycophenolate mofetil	8 (18%)	4 (13%)	4 (31%)	0·21
Azathioprine	3 (7%)	2 (6%)	1 (8%)	1·00
Ciclosporin A	2 (5%)	1 (3%)	1 (8%)	0·51
Cyclophosphamide	12 (27%)	8 (26%)	4 (31%)	0·73
HSFC B-cell counts				
Baseline				
Naive B cells, cells per µL	78·0 (38·9–137·7)	74·6 (38·0–120·0)	100·0 (41·8–193·3)	0·37
Memory B cells, cells per µL	10·5 (5·3–31·0)	11·0 (4·5–35·0)	9·90 (6·3–15·0)	0·93
Plasmablasts, cells per µL	0·7 (0·0–2·0)	0·3 (0·0–1·5)	2·0 (0·4–3·7)	0·026
2 weeks after rituximab infusion				
Naive B cells, cells per µL	0	0	0·0 (0·0–0·5)	0·24
Complete depletion of naive B cells	35 (80%)	26 (84%)	9 (69%)	0·41
Memory B cells, cells per µL	0	0	0·0 (0·0–0·2)	0·045
Complete depletion of memory B cells	38 (86%)	29 (94%)	9 (69%)	0·053
Plasmablasts, cells per µL	0·0 (0·0–0·7)	0	0·6 (0·0–0·7)	<0·0001
Complete depletion of plasmablasts	33 (75%)	29 (94%)	4 (31%)	<0·0001
Count less than 5 cells per µL	43 (98%)	31 (100%)	12 (92%)	0·30

Data are median (IQR) or n (%). *Of 12 patients with antisynthetase syndrome, nine had anti-Jo-1 antibodies, two had anti-PL-7 antibodies, and one had anti-PL-12 antibodies. †Reasons for discontinuation included treatment non-response (methotrexate n=8, mycophenolate mofetil n=6, cyclophosphamide n=1, azathioprine n=2, ciclosporin A n=2), intolerance (methotrexate n=8, mycophenolate mofetil n=2, cyclophosphamide n=1, azathioprine n=1), and reaching the maximum cumulative dose tolerated (cyclophosphamide n=10).

Table 1: **Patients' characteristics and comparison between patients responding and not responding to the first rituximab cycle**

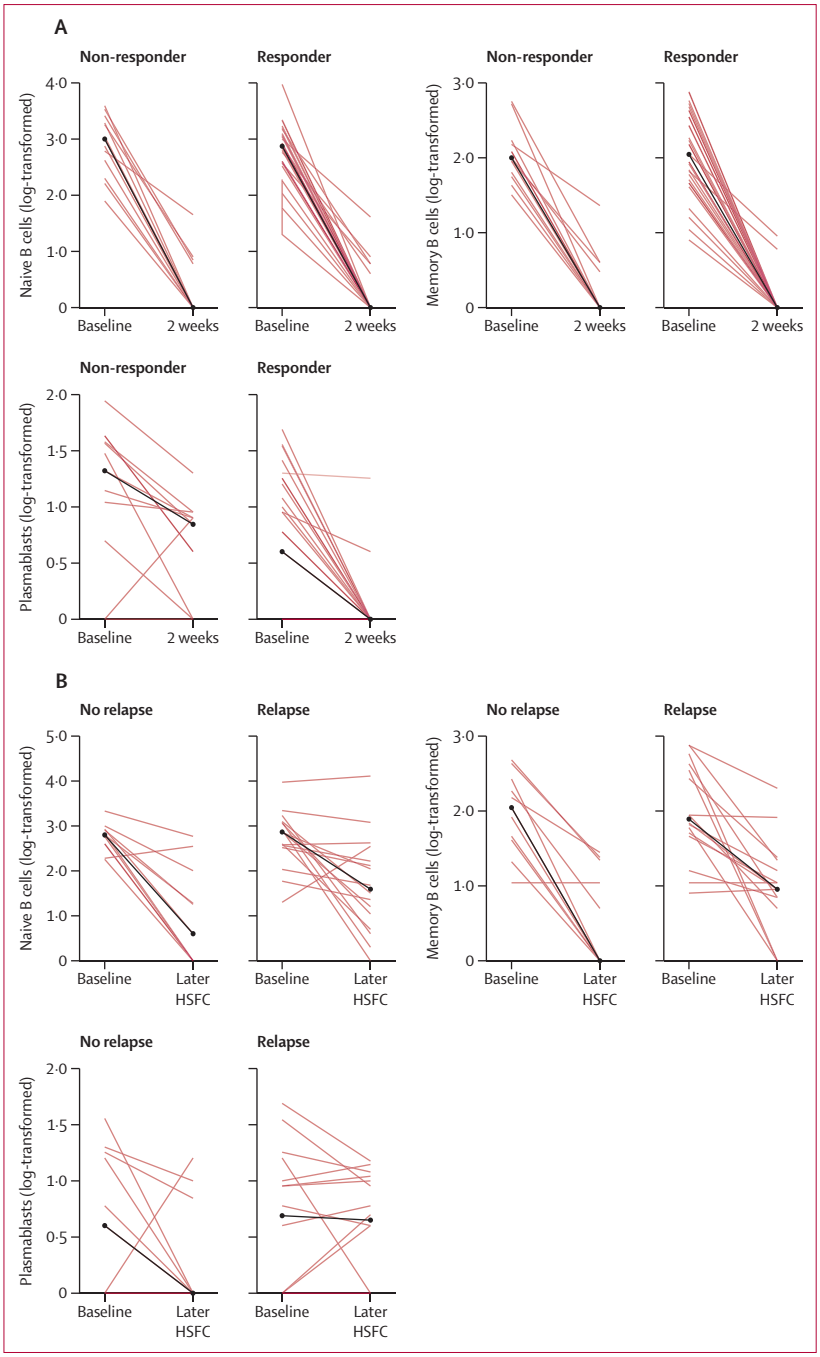


***Figure 1*: Individual longitudinal trajectories of circulating B-cell subsets assessed by highly sensitive flow cytometry**

Black lines show mean trajectories. (A) Trajectories from baseline to the 2-week timepoint, stratified by clinical response to the first rituximab cycle. (B) Trajectories from baseline to the later HSFC timepoint, stratified by relapse status in initial responders.

were receiving methotrexate, and four (9%) were receiving intravenous immunoglobulins. Such treatments remained stable over the follow-up period. Indications for rituximab included active skeletal muscle involvement in 19 (43%) patients, interstitial lung disease in 13 (30%), cutaneous involvement in 11 (25%), and arthritis in nine (21%)—patients could have more than one treatment indication. Disease activity parameters at baseline are reported in table 1.

Baseline HSFC counts were 78·0 cells per µL (IQR 38·9–137·7) for naive B cells, 10·5 cells per µL (5·3–31·0) for memory B cells, and 0·7 cells per µL (0·0–2·0) for plasmablasts (table 1). No correlation was found between baseline counts of circulating B cells and disease activity indices. 2 weeks after the first rituximab infusion, all counts significantly decreased (naive B cells 0·0 cells per µL [IQR 0·0–0·0], memory B cells 0·0 cells per µL [0·0–0·0], and plasmablasts 0·0 cells per µL [0·0–0·7]; all p<0·0001; figure 1, table 1).

At the time of follow-up assessment (median 4 months [IQR 4–5]), clinical response was observed in 31 (70%) patients and no response was observed in 13 (30%) patients. The total improvement score was higher in responders than non-responders (median: 50·0 [IQR 45·0–55·0] *vs* 17·5 [10·0–30·0]; p<0·0001; table 2). 27 (87%) of 31 responders had a moderate response and four (13%) had a major response; six (46%) of 13 non-responders had minimal improvement and seven (54%) had no improvement. On HSFC, baseline plasmablast counts were significantly lower in responders than non-responders (0·3 cells per µL [IQR 0·0–1·5] *vs* 2·0 cells per µL [0·4–3·7]; p=0·026), whereas no differences were observed for naive and memory B cells. Despite the small number of patients, anti-SRP was evident only in non-responders (p=0·083; table 1).

Using the conventional flow cytometry cutoff for total B cells, 31 (100%) responders and 12 (92%) of non-responders reported complete depletion at 2 weeks, without a statistically significant difference between groups. By contrast, the HSFC cutoff (<0·1 cells per µL) and subset phenotyping provided greater and more informative discrimination. Despite a zero-inflated distribution, memory B-cell counts (0·0 cells per µL [IQR 0·0–0·0] *vs* 0·0 cells per µL [IQR 0·0–0·2]; p=0·045) and plasmablast counts (0·0 cells per µL [0·0–0·0] *vs* 0·6 cells per µL [0·0–0·7]; p<0·0001) at 2 weeks were lower in responders than non-responders. At 2 weeks, complete depletion of plasmablasts was observed more frequently in responders than non-responders (29 [94%] *vs* four [31%]; p<0·0001), with no significant difference for memory B-cell depletion (29 [94%] *vs* nine [69%]; p=0·053; table 1; figure 1). No difference was observed between the two groups for naive B-cell counts and depletion at 2 weeks.

Multivariable models were fitted to examine the association of baseline plasmablast counts, 2-week memory B-cell and plasmablast counts, and complete depletion of these subsets at 2 weeks, with clinical response (table 3). Higher 2-week plasmablast counts were associated with a lower likelihood of subsequent response (odds ratio [OR] 0·03 [95% CI 0·00–0·23]; p=0·0002), consistent across different sets of covariates. Complete depletion of memory B cells (8·68 [1·13–82·80];

	Whole cohort (n=44)	Responders (n=31)	Non-responders (n=13)	p value
Physician global disease activity (0–10), baseline	7·0 (7·0–8·0)	7·0 (7·0–8·0)	7·0 (7·0–8·0)	0·82
Physician global disease activity (0–10), follow-up	4·0 (3·0–6·0)	3·5 (2·0–4·0)	6·0 (6·0–7·0)	<0·0001
Patient global disease activity (0–10), baseline	7·5 (7·0–8·0)	7·5 (7·0–8·0)	7·5 (7·0–8·0)	0·61
Patient global disease activity (0–10), follow-up	4·0 (3·5–6·0)	4·0 (3·0–5·0)	7·0 (6·5–7·0)	<0·0001
Manual muscle testing (0–150), baseline	144·0 (134·0–149·0)	144·0 (132·0–150·0)	140·0 (136·0–146·0)	0·50
Manual muscle testing (0–150), follow-up	146·0 (140·0–150·0)	148·0 (144·0–150·0)	144·0 (136·0–146·0)	0·036
Health assessment questionnaire (0–3), baseline	1·00 (0·50–1·60)	0·90 (0·30–1·60)	1·10 (0·70–1·88)	0·26
Health assessment questionnaire (0–3), follow-up	0·70 (0·30–1·15)	0·50 (0·25–1·00)	1·10 (0·70–1·80)	0·019
Extramuscular activity (0–10), baseline	7·0 (5·0–8·0)	6·0 (4·5–8·0)	7·0 (6·0–8·0)	0·35
Extramuscular activity (0–10), follow up	3·0 (2·0–5·0)	3·0 (1·0–3·5)	7·0 (5·0–8·0)	0·0003
Creatine kinase (units per L), baseline	130 (62–603)	118 (68–515)	141 (56–690)	0·88
Creatine kinase (units per L), follow-up	84 (56–207)	75 (55–214)	127 (72–200)	0·43
Total improvement score	46·3 (32·5–52·5)	50·0 (45·0–55·0)	17·5 (10·0–30·0)	<0·0001

Total improvement score determined per European Alliance of Associations for Rheumatology recommendations.

Table 2: **Core items of the total improvement score at baseline and at follow-up in patients responding and not responding to rituximab**

	Demographics only		Clinical-adjusted		SRP-adjusted	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Baseline counts						
Age	1·00 (0·96–1·05)	0·95	1·01 (0·96–1·05)	0·80	1·00 (0·95–1·04)	0·86
Male sex	2·58 (0·65–12·67)	0·18	2·61 (0·63–13·53)	0·19	2·06 (0·50–10·29)	0·33
Plasmablasts, cells per µL	0·35 (0·10–1·01)	0·052	0·38 (0·11–1·12)	0·079	0·34 (0·10–1·00)	0·050
Mechanic's hands	..	..	0·25 (0·05–1·08)	0·063	..	..
Anti-SRP	..	..	..	..	0·10 (0·01–1·54)	0·10
Counts 2 weeks after rituximab infusion						
Age	1·00 (0·94–1·05)	0·92	1·00 (0·94–1·06)	0·97	0·98 (0·92–1·04)	0·54
Male sex	1·85 (0·32–12·69)	0·49	1·99 (0·34–15·0)	0·45	1·22 (0·17–9·20)	0·84
Memory B cells, cells per µL	0·13 (0·01–1·06)	0·056	0·15 (0·01–1·65)	0·12	0·11 (0·01–1·01)	0·051
Plasmablasts, cells per µL	0·03 (0·00–0·23)	0·0002	0·04 (0·00–0·25)	0·0004	0·02 (0·00–0·17)	<0·0001
Mechanic's hands	..	..	0·28 (0·04–1·64)	0·15	..	..
Anti-SRP	..	..	..	..	0·03 (0·00–0·92)	0·045
Complete depletion 2 weeks after rituximab infusion						
Age	1·00 (0·95–1·05)	0·93	1·01 (0·95–1·06)	0·81	0·98 (0·92–1·04)	0·56
Male sex	2·26 (0·36–18·18)	0·39	2·43 (0·34–24·80)	0·38	1·61 (0·20–14·57)	0·65
Depletion of memory B cells	8·68 (1·13–82·80)	0·038	9·24 (0·95–126·00)	0·056	13·54 (1·41–190·00)	0·024
Depletion of plasmablasts	25·20 (4·61–214·54)	<0·0001	26·51 (4·48–284·00)	0·0001	45·92 (6·50–689·00)	0·0001
Mechanic's hands	..	..	0·22 (0·02–1·50)	0·12	..	..
Anti-SRP	..	..	..	..	0·02 (0·00–0·70)	0·032

OR=odds ratio. SRP=signal recognition particle.

Table 3: **Multivariable logistic regression models (Firth's penalised likelihood) assessing associations between covariates and the clinical response outcome**

p=0·038) and plasmablasts (25·20 [4·61–214·54]; p<0·0001) at 2 weeks was positively associated with subsequent response. These associations were corroborated independently from concurrent glucocorticoid and immunosuppressive treatments (table 4).

Among covariates other than HSFC parameters, the likelihood of response was inversely associated with anti-SRP antibodies (OR 0·02 [95% CI 0·00–0·70]; p=0·032). In the two patients with anti-SRP positivity, one had a complete depletion of all B-cell lineages at 2 weeks, whereas the other did not have depletion of memory B cells and plasmablasts (figure 2).

Of 31 responders, 27 patients had subsequent HSFC follow-up data, assessed at a median of 12 months (IQR 8–16) after the first rituximab infusion. 11 (41%) of 27 patients maintained their response and 16 (59%) relapsed within 3 months of the HSFC follow-up assessment. Compared with baseline levels, counts

	Glucocorticoids-adjusted		Immunosuppressants-adjusted	
	OR (95% CI)	p value	OR (95% CI)	p value
Baseline counts				
Age	0·99 (0·95–1·04)	0·76	1·00 (0·96–1·05)	0·97
Male sex	3·09 (0·73–17·1)	0·13	2·81 (0·68–14·9)	0·16
Plasmablasts, cells per µL	0·35 (0·10–1·03)	0·056	0·26 (0·062–0·82)	0·021
Glucocorticoids*	0·35 (0·071–1·44)	0·15	..	..
Immunosuppressants†	..	..	0·35 (0·064–1·53)	0·17
Counts 2 weeks after rituximab infusion				
Age	0·99 (0·94–1·04)	0·67	1·00 (0·94–1·05)	0·93
Male sex	2·15 (0·36–17·20)	0·40	1·83 (0·32–12·7)	0·50
Memory B cells, cells per µL	0·12 (0·01–0·99)	0·049	0·14 (0·01–1·13)	0·065
Plasmablasts, cells per µL	0·03 (0·00–0·25)	0·0003	0·04 (0·00–0·25)	0·0002
Glucocorticoids*	0·31 (0·04–1·74)	0·19	..	..
Immunosuppressants†	..	..	0·90 (0·14–5·35)	0·91
Complete depletion 2 weeks after rituximab infusion				
Age	0·99 (0·93–1·04)	0·60	1·00 (0·94–1·05)	0·91
Male sex	2·48 (0·36–25·20)	0·36	2·09 (0·34–17·10)	0·43
Depletion of memory B-cells	8·53 (1·03–102·00)	0·047	8·18 (1·09–76·80)	0·041
Depletion of plasmablasts	23·90 (4·29–227·00)	0·0001	23·0 (4·31–193·00)	0·0001
Glucocorticoids*	0·28 (0·03–1·70)	0·17	..	..
Immunosuppressants†	..	..	1·18 (0·18–7·97)	0·86

HSFC=highly sensitive flow cytometry. OR=odds ratio. *≥10 mg/day of prednisolone equivalent. †Mycophenolate mofetil, azathioprine, or methotrexate.

Table 4: **Multivariable logistic regression models (Firth's penalised likelihood) assessing associations between HSFC-derived B-cell counts and the clinical response outcome**

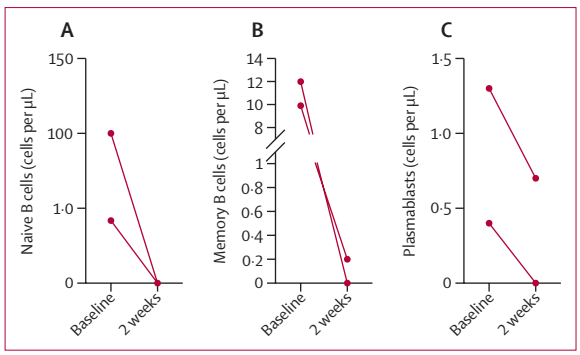


Figure 2**: Baseline-to-2 week trajectories of naive B cells, memory B cells, and plasmablasts in the two patients with anti-SRP positivity who did not have a clinical response**
Naive B cells (A), memory B cells (B), and plasmablasts (C).

remained reduced across all B-cell subsets, and persistent complete depletion at second HSFC follow-up was observed in six (22%) patients for naive B cells, nine (33%) for memory B cells, and 14 (52%) for plasmablasts. Patients who relapsed were less likely to still have complete depletion at follow-up. Complete depletion of naive B cells was still observed in one (6%) of 16 patients who relapsed versus five (46%) of 11 patients who did not relapse (p=0·027). Complete depletion of memory B cells was present in three (19%) patients who relapsed versus six (55%) patients who did not relapse (p=0·097). Complete depletion of plasmablasts was seen in six (38%) patients who relapsed versus eight (73%) patients who did not relapse (p=0·072). Baseline characteristics and baseline and 2-week HSFC B-cell subset counts were similar between those who relapsed and those who did not (figure 2; appendix p 3). Overall, 29 responders received at least one more rituximab cycle. One patient—who did not respond to rituximab—died during follow-up.

Discussion

Despite some favourable evidence, the role of rituximab in idiopathic inflammatory myopathies remains incompletely defined. Herein, in rituximab-treated myositis, HSFC-based quantification of circulating B-cell subsets at baseline and after 2 weeks was associated with clinical response. In keeping with the minimal residual disease concept, undetectable memory B cells and plasmablasts at 2 weeks after the first rituximab infusion predated subsequent clinical response; instead, failure to deplete (ie, detectable minimal residual disease) was associated with an unfavourable outcome. B-cell reconstitution (>0·1 cells per µL) during follow-up was linked to increased risk of relapse in initial responders. Although these associations were evident using HSFC cutoffs for minimal residual disease, the conventional flow cytometry cutoffs (<5 total B cells per µL) did not identify similar associations. These findings identify an association between the depth of peripheral B-cell depletion and rituximab responsiveness and support a potential role for HSFC in guiding a more individualised use of B-cell depleting strategies in myositis, extending previous evidence in rheumatoid arthritis, SLE, and antineutrophil cytoplasmic antibody-associated vasculitis.[18–21] Since the induction and maintenance of complete depletion for selected B-lineage subsets was associated with outcomes, HSFC monitoring of residual circulating B-cell subsets could help inform both the need and timing to switch from rituximab to more advanced depleting therapies, such as type II monoclonal antibodies (ie, obinutuzumab, which leads to better complement-mediated and cell-mediated B-cell cytotoxicity), bispecific T-cell engagers, or CAR T cells. This approach is something that is essentially absent in the rheumatology literature compared with haematology.

Overall, response to rituximab was observed in 31 (70%) of 44 patients with idiopathic inflammatory myopathies. No baseline clinical or demographic feature was associated with clinical response. B cells were detectable in most patients before rituximab administration, with naive B cells representing the most abundant population and plasmablasts the least abundant. Pre-rituximab plasmablast counts were significantly lower in subsequent responders than in non-responders, in line with previous observations in rheumatoid arthritis.[18,19]

By linking clinical response to B-cell depletion, these findings could help to explain the observed heterogeneity between negative clinical trials versus positive observational data of rituximab in patients with idiopathic inflammatory myopathies.[11,26,27] HSFC enumeration of circulating B-cell subsets at 2 weeks after the first rituximab infusion was strongly associated with subsequent clinical response. At this early timepoint, lower absolute plasmablast counts were independently associated with clinical response; no significant difference was seen for memory B cells or naive B cells. Crucially, complete depletion (<0·1 cells per µL) of plasmablasts and memory B cells was associated with response, whereas conventional thresholds (<5 cells per µL) did not provide discrimination, highlighting the role of highly sensitive techniques in defining minimal residual disease. These observations are consistent with data in patients with rheumatoid arthritis,[18] Sjögren's disease,[28] and SLE[20]—however, later timepoints could be more informative.

Response was not observed in the two patients with anti-SRP antibodies, a finding that should be interpreted with caution due to sparse data. Nonetheless, these observations might align with reports of anti-SRP titres failing to decrease after treatment with rituximab, as opposed to other autoantibodies,[29] and might support targeting CD20-negative B-cell lineages, such as long-lived plasma cells,[30] in anti-SRP-positive myositis.

Of the 27 responders with subsequent HSFC follow-up data, 16 (59%) had a relapse during follow-up. Although the study was not designed for this comparison, B-cell reconstitution as high as HSFC-detectable minimal residual disease (>0·1 cells per µL) was reported more often in patients who relapsed compared with those who did not relapse, similar to rheumatoid arthritis[19,31] and SLE.[20,32] Although the interval between ascertained clinical response and the first subsequent HSFC assessment was heterogeneous, reflecting routine clinical practice, these findings raise the possibility that longitudinal monitoring for minimal residual disease after B-cell depleting therapies could inform relapse risk and support timely retreatment or escalation strategies—including dose intensification[33] or switching to other B cell-directed or plasma cell-directed targets[12,13]—before an overt clinical flare.

The clinical applicability of HSFC warrants consideration. High-sensitivity B-cell subset monitoring is achievable with a minimum six-colour panel, a configuration available on most modern flow cytometers in centres where B-cell depleting therapies are routinely administered. Reproducible detection of B-cell subsets at the threshold used in this study requires acquisition of at least 500 000 leucocyte events, which is readily achievable on most six-colour instruments in clinical use. Standardisation of HSFC protocols across centres, including harmonisation of panel design, acquisition thresholds, and gating strategies, will be a prerequisite for its broader adoption and multicentre trials.

These findings provide proof-of-concept that HSFC is clinically informative to guide treatment in patients with idiopathic inflammatory myopathies. The associations between early B-cell subset depletion and subsequent clinical response suggest that HSFC could serve as a pharmacodynamic biomarker to guide precision treatment escalation. Specifically, early persistence of minimal residual memory B cells or plasmablasts could identify patients unlikely to respond to rituximab, supporting timely escalation to more potent B cell-directed or plasma cell-directed strategies. Sustained undetectable subsets during follow-up could identify patients in whom retreatment can be safely deferred. Prospective validation in independent cohorts is required for implementation of HSFC in routine practice.

This study has several limitations. First, its retrospective, single-centre design limits generalisability. Although HSFC is routinely performed as part of the assessment of patients receiving rituximab at the study centre, only patients with HSFC data were eligible for inclusion, potentially introducing some degree of selection bias. Patients received rituximab according to clinician's judgement and for different indications; thus, despite baseline disease activity being homogeneous, patients were not selected through pre-established criteria. These considerations support the need for external and prospective validation in independent cohorts and, potentially, randomised clinical trials assessing early B-cell depletion as a surrogate endpoint of subsequent clinical response. The correlation between B-cell populations and the kinetics of serum autoantibodies before and after rituximab administration should be also explored. Only one patient—who did not respond to rituximab—died during follow-up, potentially introducing some bias and preventing the assessment of any link between depletion or repopulation and survival. Second, because of the limited sample size, multivariable adjustment was necessarily restricted, and prespecified models were fitted (including combinations of demographic, clinical, and autoantibody variables) with a limited number of covariates. Firth's penalised likelihood was used to mitigate sparse-data bias, but we could not simultaneously adjust for all potentially relevant confounders (autoantibodies, organ involvement, disease phenotype, and treatments). This approach mitigates the risk of overfitting, but some degree of residual confounding should be still accounted for. Therefore, the findings should be interpreted carefully and require further validation. Third, associations with disease relapse could only be explored using univariable comparisons and no fixed follow-up timepoint was defined. Evidence of disease relapse could have prompted HSFC evaluation before routine monitoring, further contributing to bias. Analyses of relapse are also susceptible to time-related biases, including immortal time bias, as patients had to remain relapse-free up to the time at which relapse could be ascertained. Thus, the analysis of disease relapse must be interpreted carefully as

purely descriptive and explorative. Prospective studies with standardised follow-up and time-to-event endpoints are needed. Fourth, the cohort comprised different subsets of patients with idiopathic inflammatory myopathies, with approximately half of patients having dermatomyositis and a substantial proportion having antisynthetase syndrome; however, there were no patients with inclusion body myositis—a distinct pathophenotype of inflammatory myopathy. As a result, the primary response endpoint was analysed in aggregate, and domain-specific stratification was not feasible due to sample size. Although the approach was in line with previous basket trials and observational analyses, future studies should focus on larger cohorts stratified according to clinical and autoantibody labels. Moreover, most rituximab responders had a moderate improvement, preventing stable comparisons between HSFC circulating B cells in patients with moderate versus major response. Finally, people with lived experience were not involved in the design, conduct, interpretation, or dissemination of this study.

In conclusion, we investigated the clinical significance of minimal residual disease concept in patients with idiopathic inflammatory myopathies treated with rituximab, based on HSFC B-cell subset assessment. Compared with conventional flow cytometry cutoffs, complete memory B cell and plasmablast depletion at 2 weeks after the first rituximab infusion was associated with subsequent clinical response. By contrast, minimal residual disease at 2 weeks was negatively associated with clinical response. Longitudinal monitoring of initial responders further suggested that disease relapses might more frequent when minimal residual disease was detected during follow-up. Although these findings require prospective validation, combining HSFC-defined minimal residual disease assessment with autoantibody profiling could help to uncover the pathogenic B-lineage programs underlying myositis immunopathology across disease subsets and provide a valuable framework for precision selection and escalation of more potent B-cell depletion strategies in patients who do not respond or who relapse.

Contributors

Conceptualisation: AT, AR, DMG. Data curation: AT, FT, AR, and DMG. Methodology: AT, EV, AR, and DMG. Investigation: AT, FT, AR, and DMG. Writing—original draft: AT and DMG. All authors contributed to review and editing, had full access to all study data, and had final responsibility for the decision to submit for publication. AT, FT, and DMG directly accessed and verified the underlying data reported in the manuscript, and all authors have approved the final version of the manuscript.

Declaration of interests

All authors declare no competing interests.

Data sharing

Data are available upon reasonable request to the corresponding author.

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