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Title
Repeat cycles of rituximab on clinical relapse in ANCA-associated vasculitis: identifying B cell biomarkers for relapse to guide retreatment decisions

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ABSTRACT

Objective: To assess clinical and B cell biomarkers to predict relapse after rituximab in ANCA-associated vasculitis (AAV) using retreatment on clinical relapse strategy.

Methods: 35 patients with AAV received treatment with 2x1000mg rituximab, repeated on clinical relapse (up to 5 cycles). Disease activity was assessed by Birmingham Vasculitis Activity Score and peripheral B cell subsets using highly sensitive flow cytometry (HSFC) as previously described; both performed at baseline and every 3 months.

Results: Response rates were high: >83%, with median time-to-relapse of 82 weeks for Cycle 1 (C1) and >54 weeks for all cycles. Prior to rituximab, AAV was characterised by naïve B-lymphopenia compared to healthy controls. This dysregulation was more marked in patients with raised CRP ($p < 0.05$). In C1, no clinical feature predicted relapse. However, repopulation of naïve B cell at 6 months was associated with a reduced risk of relapse (HR: 0.326, 95% 0.114-0.930, p=0.036). Relapse rates at 12 and 18 months were 0% and 14% with naïve repopulation at 6 months, and 31% and 54% without naïve repopulation.

Conclusion: Responses to B cell depletion therapy are long-lasting and relapse post-treatment may be predicted by absence of naïve B cell repopulation at 6 months. Naive B-lymphopenia may be a biomarker of disease activity in AAV.

(205 words)

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INTRODUCTION

B cells are central to the pathogenesis of AAV(1) and a single cycle of B cell depletion with rituximab is effective in inducing remission(2, 3). However, the optimal long-term strategy for rituximab-treated patients has not yet been established.

Experience with rituximab in other autoimmune diseases indicates that the duration of response to each cycle is variable, and the minimum necessary retreatment interval will also therefore vary between patients (4, 5). In rheumatoid arthritis (RA), repeat cycles are often given on clinical relapse. This approach may be riskier in AAV since relapses may cause life- or organ-threatening disease. An alternative approach is to use pre-emptive treatment either based on reconstitution of B cells or fixed-intervals (6, 7). However, flares in the absence of peripheral B lymphocyte have been observed in the former (8) while the latter may lead to hypogammaglobulinaemia and serious infection (9, 10). Alternatively, low dose rituximab (500mg) can be given every 6 months as per MAINRITSAN regimen; although the comparative long term of this approach is not yet known(11). Therefore, biomarkers that could guide these decisions would be valuable.

In RA and systemic lupus erythematosus (SLE), analysis of B cell subsets using Highly Sensitive Flow Cytometry (HSFC) predicted clinical response and relapse (4, 12). The objective of this study was to assess clinical and B cell biomarkers to predict relapse in AAV patients with response to rituximab.

PATIENTS AND METHODS

Details about the methodology can be found in supplementary files.

Patients

Data from all patients (>18 years old) with AAV (13) treated with rituximab in our unit between January 2006 and September 2013 were analysed retrospectively. Leeds (West) Research Ethics Committee confirmed that ethical approval was not required in accordance with the UK National Health Service Research Ethics Committee guidelines.

Treatment Protocol

All patients received a first cycle of therapy consisting of 100 mg of methylprednisolone and 1000 mg of rituximab on days 1 and 14, with a course of prednisolone at 60 mg daily on days

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1–7 and 30 mg daily on days 8–14. Further cycles consisted of the same regimen repeated on clinical relapse (defined below). Continuation of a stable dose or reduction of concomitant IS including oral corticosteroid was left to investigator's discretion with the aim to stop glucocorticoid if remission is achieved at 6 months. Concomitant cyclophosphamide was not used.

Clinical Data and Outcomes

Disease activity was assessed at baseline and every 3 months post-therapy using Birmingham Vasculitis Activity Score (BVAS 3.0) (14) without knowledge of B cell results. Complete response (CR) was defined as BVAS = 0 while partial response (PR) was defined using an existing arbitrary definition as 50% improvement in BVAS from baseline, both at Week 26(15). Relapse was defined as an increase in the BVAS ≥ 1. Vasculitis Damage Index (VDI) was recorded every 6 months post-therapy.

Laboratory Assessments

Peripheral blood B cell subsets were measured using HSFC as previously described at week 0, 6 and 26 without knowledge of clinical status other than time since rituximab(16)(see supplementary file S1). Complete B cell depletion was defined as counts ≤ 0.0001 x 10^9 cells/L and repopulation as counts >0.0001 x 10^9 cells/L. 12 previously recruited healthy controls were used to measure normal B cell subsets. Comparison was also made to our previous data in rituximab-treated RA (n=95) and SLE (n=44) cohorts (4, 16).

RESULTS

Patient Characteristics

37 consecutive patients with active severe AAV received treatment with rituximab. 35 patients were included in the analysis (162 patient-years follow-up) as two patients subsequently followed a pre-emptive re-treatment strategy guided by rising ANCA levels. Baseline characteristics are described in supplementary Table S1.

Treatment Characteristics

110 cycles of rituximab were administered. Median (IQR) weeks-to-relapse for Cycles 1-5 (C1-5) were 82 (58-123), 71 (52-77), 58 (50-70), 54 (44-80) and 59 (44-78) respectively.

Prior to the first rituximab infusion, 18 patients (51%) were already receiving a conventional oral IS: methotrexate (MTX) = 7, azathioprine (AZT) = 6 and mycophenolate mofetil (MMF)

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= 5. At the last follow-up (data last updated), 7 patients (20%) were on rituximab monotherapy for maintenance, most with limited disease (ENT involvement).

Clinical Response

Response rates for C1-5 were 33/35 (94%), 28/28 (100%), 17/20 (85%), 11/13 (85%) and 5/6 (83%) respectively. Further details regarding the pattern of clinical response, relapse and damage are given in Figure 1 and the supplementary file.

Predictors of relapse: Baseline characteristics

Using univariate cox-regression, only baseline memory B cell number was a significant predictor of relapse (HR: 1.014, 95% CI [1.001-1.028]), p=0.040 (Supplementary Table S2). However, this relationship was not found in subsequent cycles. Multivariate analysis was not performed since there was only 1 baseline significant predictor and due the size of the study population. No clinical feature predicted relapse (Table S2).

Before rituximab, AAV was characterised by naïve and memory B-lymphopenia compared to healthy controls, all p<0.05 (Figure 2(A)). These features were more marked in patients with raised CRP (Figure 2(A)). Naïve B cell numbers at 6 months were lower in AAV compared to RA or SLE (Figure 2(B)).

Predictors of relapse: post-rituximab therapy

After C1, there was only a weak trend to longer time-to-relapse in patients with CR compared to PR (Log-rank (Mantel–Cox) test, x^2 = 1.675, df = 1, p=0.196). Time-to-relapse for C1 and 2 showed moderate correlation (r=0.490, p=0.020). However, duration of C1 alone did not appear clinically useful in estimating duration of C2; the second cycle was >12 weeks longer than the first in 30% of cases, and >12 weeks shorter than the first in 45% of cases.

At 6 months, B cells were detectable in 70% of the patients. This time point preceded all relapses. In order to analyse the effect of B cell repopulation and relapse, we compared time to relapse in patients according to presence or absence of each subset (naïve, memory, plasmablast).

We found a significant association between repopulation of naïve B cells at 6 months and time to relapse (p=0.010, Figure 3(D)), but no association between memory B cell (p=0.399) or plasmablast repopulation (p=0.262) and relapse. This is in marked contrast to findings in

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SLE (4). In order to better understand these results, we compared baseline clinical characteristics of patients according to presence or absence of naïve B cells at 6 months. Patients without detectable naïve B cells at 6 months had lower naïve B cells and significantly higher CRP (p=0.015) at baseline, but no difference in age, disease duration, BVAS 3.0, PR3 or MPO titres. Relapse rates for this group of patients at 12 and 18 months were 31% and 54% respectively.

Of patients with detectable naïve B cells at 6 months, relapse rates at 12 and 18 months were 0% and 14% respectively. The same trend was observed in subsequent cycles; C2 (n=12) and C3 (n=8): 0% relapse rates for both at 12 months after each cycle.

We further explored the relationship between repopulation of naïve ($CD19^+CD27^-$) cells and later relapse, in a subset of patients (n=6). We divided these cells into $CD19^+CD27^-CD38^-$ "mature naïve B cells" and $CD19^+CD27^-CD38^+$ "transitional B cells." We found two distinct groups of transitional B cell repopulation: low frequency (1.5-5.0%) and high frequency (20.8-84.3%). Due to sample size, we were unable to confirm the association of these groups with duration of response.

DISCUSSION

In this study, we identified a B cell biomarker for relapse prediction using a "retreatment on clinical relapse" strategy.

Active AAV was characterised by naïve lymphopenia compared to healthy controls, RA and SLE patients at baseline. The effect of immunosuppressive therapy on B cell homeostasis (17) was not studied directly. However, there was no significant difference in lymphocyte count or relative B cell numbers between patients who had received initial remission induction with cyclophosphamide versus rituximab alone, or comparing patients with or without oral IS at baseline. (Table S1 and Figure S1). Moreover, the dysregulation of naïve B cells was more marked in patients with more severe systemic inflammation (high CRP). This observation suggests that naïve lymphopenia may be a biomarker of disease-associated B cell activity.

Patients with early relapse failed to repopulate with naïve B cells. This differs our results in SLE (4). In SLE, we observed sustained suppression of memory B cells and plasmablasts despite repopulation of naïve B cells that predicted longer responses. Undetectable naïve B

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cells at 26 weeks may be an early indication of the recurrence of the naïve lymphopenia that characterised more severe disease before rituximab, and therefore serves as an early sign of disease-associated B cell activity.

Alternatively, this subset might contain a population of regulatory B cells (Breg) that help to maintain remission. These have previously been identified within transitional B cell subsets, $CD19^+CD24^{hi}CD38^{hi}27^-$ cells[18]. Wilde et al.[19] demonstrated that interleukin (IL-10) competency is diminished during active and remission in AAV patients. Todd et al.[20] concurred, but also reported an increase in frequency of Breg following rituximab, still present 5 years after treatment. This intriguing hypothesis needs to be evaluated in a prospective cohort study.

Regardless of the mechanistic explanation, these results suggest that evaluation of naïve B cell numbers in very early repopulation using HSFC may have value in guiding retreatment decisions for the most effective, efficient use of rituximab in AAV. Patients with undetectable naïve B cells at 6 months may be suited for earlier retreatment due to 30% relapse rate observed at 12 months. We conclude that naïve lymphopenia may be a B cell specific marker of disease activity in AAV. This warrants validation in larger cohorts.

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LEGENDS TO FIGURES

Figure 1: Outcome measures with successive rituximab treatment cycles. BVAS: Birmingham Vasculitis Activity Score, C: cycle, 6-mo: 6 months. (A) The mean BVAS on clinical relapse for each cycle was significantly lower than the mean BVAS at original baseline ($p<0.001$ for all comparisons with Bonferroni correction using adjusted alpha levels of 0.0125). (B) The median daily dose of oral prednisolone recorded at 6 months of each cycle was significantly lower than the baseline oral prednisolone prior to rituximab; $p<0.001$ after adjusting for Bonferroni correction with adjusted alpha level of 0.0125. (C) Percentage of patient who had either complete response, partial response or no clinical response in C1 of rituximab. There was a weak trend to association between incomplete peripheral B cell depletion at 6 weeks and clinical non-response at 6 months ($p=0.187$). (D) Repeat cycles of rituximab on clinical relapse strategy did not result in significant progressive deterioration in IgG level compared to baseline IgG ($p = 0.760$ after adjusting for Bonferroni correction with adjusted alpha level of 0.0125)

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Figure 2: Peripheral B cell subsets prior to rituximab and along the course of rituximab treatment and comparison with other diseases. AAV: ANCA-Associated Vasculitis, CRP: C-reactive protein, HC: healthy controls, RA: rheumatoid arthritis and SLE: systemic lupus erythematosus. (A) Prior to rituximab, active AAV was characterised by naïve- and memory-b lymphopenia compared to healthy controls at baseline. This dysregulation was more marked in patients with severe systemic inflammation; raised CRP (CRP>10mg/dL). (B(i)) AAV patients exhibited lower naïve B cells before and 6 months after rituximab compared to RA and SLE patients. (B(ii)) Early repopulation of naïve B cells at 6 months repeated at larger scale to help compare medians and IQRs in these diseases.

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Figure 3: Relapse free survival according to (A) clinical response at 6 months, (B) concomitant immunosuppressive therapy, (C) baseline serum CRP concentration and (D) naïve B cell repopulation at 6 months. There was no association of achieving complete remission at 6 months, concomitant use of immunosuppressant and raised C-reactive protein (CRP) at baseline with time to relapse. Figure 3(D): Early repopulation of naïve B cells at 6 months was associated with later clinical relapse.

Competing Interests:

Contributorship Statement:

Dr Md Yusof MY, Dr Vital EM and Prof Emery P: Substantial contributions to the conception or design of the work, or the acquisition, analysis or interpretation of data, drafting the work or revising it critically for important intellectual content, final approval of the version published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Dr Das S and Dr Dass S: Substantial contributions to the conception or design of the work, or the acquisition, analysis or interpretation of data, drafting the work or revising it critically for important intellectual content and final approval of the version published

Dr Arumugakani G, Dr Savic S and Dr Rawstron A: Substantial contributions to the conception or design of the work, or the acquisition, analysis or interpretation of data and final approval of the version published

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Leeds (West) Research Ethics Committee confirmed that ethical approval was not required in accordance with the UK National Health Service Research Ethics Committee guidelines because all treatment decisions were made prior to evaluation of data.

Data Sharing Statement:

None

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