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Original article

Low pre-treatment B-cell counts are not a risk factor of infection in patients treated with rituximab for autoimmune diseases: An observational study

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ABSTRACT

Objectives: Rituximab (RTX) is increasingly used in patients with refractory rheumatoid arthritis (RA) and other severe autoimmune diseases (AID). In practice, many clinicians are reluctant to prescribe RTX in patients with low B-cell counts because of the presumed risk of infection. The aim of this study was therefore to investigate whether B-cell counts before treatment or retreatment with RTX predict the occurrence of infections.

Methods: Observational, single-centre study of 161 patients treated with RTX for RA and other AID at a tertiary hospital. CD19+ B-cell counts were assessed by flow cytometry and multivariate statistical analysis adjusted for various potential predictors was performed.

Results: The rate of severe infection was 5.9/100 patient-years in RA patients and 24.9 in non-RA AID ($P < 0.001$). Low B-cell counts at the time of RTX infusion were not associated with subsequent severe (HR = 0.55, $P = 0.60$) or overall infection (HR = 0.85, $P = 0.58$). Significant pre-treatment predictors of severe infection were a diagnosis other than RA (HR = 4.68, $P < 0.001$), immunoglobulin (Ig) G levels <7 g/L (HR = 2.36, $P = 0.01$), age (HR = 1.03, $P = 0.01$), and diabetes (HR = 3.61, $P = 0.01$).

Conclusions: Low B-cell counts before RTX infusion did not predict subsequent infections in this population treated with RTX for RA and other AID, therefore not supporting the practice of pre-treatment assessment of B-cells. Nevertheless, a higher risk of severe infection was confirmed for low pre-treatment IgG levels, older age, diabetes, and AID other than RA.

1. Introduction

Rituximab (RTX) induces profound depletion of B-cells through complement-mediated mechanisms, antibody-dependent cellular cytotoxicity, and apoptosis [1,2]. RTX is currently licenced by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) for use in anti-TNFα-refractory rheumatoid arthritis (RA) and severe anti-neutrophil cytoplasm antibodies (ANCA)-associated vasculitis. However, RTX is widely used off-label as an add-on therapy for a variety of other autoimmune diseases (AID) [3,4], in particular for patients with severe refractory disease or intolerant to classical immunosuppressive regimens.

The risk of infection in patients treated with RTX depends on the criteria used for defining treatment-related infections and the indication for its use, i.e. hematologic malignancies versus AID [5]. In RA, a meta-analysis of randomised placebo-controlled trials has demonstrated an incidence of severe infections of 2.3% in patients treated with RTX compared to 1.5% in the placebo group [6], and data from the French registry revealed a rate of severe infections of 5/100 patient-years [7]. Data from observational cohorts and randomized controlled trials generally show a higher risk in non-RA patients following RTX treatment, up to 22.5/100 patient-years [8–19]. Recognised risk factors for severe infections in these populations included low immunoglobulin (Ig) G levels, older age, comorbidities, extraarticular RA involvement, and concomitant immunosuppressive therapy.

Assessment of circulating B-cell counts before treatment seems to be of limited clinical value for the prediction of response to RTX in RA [20]. An open question, however, is the value of B-cell counts

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before treatment or retreatment with regards to the risk of infection. Many clinicians assess baseline counts before treatment or retreatment and seem reluctant to administer RTX if B-cells are decreased, due to the presumed risk of severe infections in these particularly vulnerable immunosuppressed patients with refractory RA and other AID. The aim of the present study was therefore to examine whether low circulating B-cell counts at the time of RTX infusion were associated or not with an increased risk of infection in a real-life setting.

2. Methods

2.1. Study design

This was a tertiary-care, single-centre historical cohort study on a registry of all patients treated with RTX for RA and other AID over the past ten years in the Rheumatology and Immunology Divisions of the University Hospitals of Geneva. The clinical and laboratory data such as B-cell counts and immunoglobulins were collected at baseline and 12 months, prior to each RTX cycle, as well as whenever available during follow-up. Data on infections and conditions predisposing to infections were collected through electronic medical chart analysis and patient questionnaires. In addition, the primary care physicians and/or rheumatologists of each patient were contacted and provided information on the occurrence of infections during follow-up. Institutional review board and Research Ethics Committee of the University Hospitals of Geneva approval (HUG REC11-186) was obtained for the study protocol and all patients provided written informed consent prior to their inclusion in the study according to the Declaration of Helsinki. Severe infections were reported to the Swiss regulatory authorities for drug safety (Swissmedic).

Primary outcome was the occurrence of severe (requiring parenteral treatment or hospitalisation and/or leading to severe disability or death) and non-severe infection (requiring antimicrobial treatment). The "at-risk" period was defined as the person-time between patient's first RTX infusion and either death or last visit. In the primary analysis, we did not restrict the "at-risk" window to a particular period ("ever at risk"), as the duration of effect with RTX may be much longer in certain patients. In accordance with the EULAR recommendations for reporting safety data of biologics registers [21], we performed several sensitivity analyses with varying duration of the "at-risk" window after the last RTX infusion (6, 12 or 18 months). Primary predictor of interest was the absolute B-cell count, assessed by flow cytometry before RTX treatment or retreatment. Complete B-cell depletion was defined as CD19-values below 0.01×10^9 cells/L, B-lymphopenia was defined as CD19-values below 90×10^6 cells/L and serum IgG levels above 7 g/L are considered normal (95% CI) in the target population (cut-off values derived from healthy blood donors).

2.2. Patients

Patients enrolled were aged >18 years, had a diagnosis of either RA or other AID, and were treated with RTX in the Rheumatology and Immunology Divisions of the University Hospitals of Geneva. The indication for RTX prescription was posed by the treating physician. Patients were excluded if no B-cell count assessment was performed.

2.3. Laboratory assays

Lymphocyte populations were enumerated by flow cytometry in EDTA-whole blood using TruCOUNT™ Tubes (Becton Dickinson, San Jose, CA, USA) and a lysed-no-washed procedure. The following monoclonal antibodies were used: CD45-PerCP, CD3-FITC, CD4-APC, CD8-PE, CD16-CD56-PE and CD19-APC (Becton Dickinson, San Jose, CA, USA). Cells were analysed by a 4-Color FACSCalibur™ (Becton Dickinson, San Jose, CA, USA) using CD45 versus side scatter of complexity (SSC) gating. BD Multiset™ software automatically calculated subset absolute counts. IgG, IgA and IgM were measured by nephelometry using BN ProSpec® nephelometer and reagents (Siemens, Marburg, Germany).

2.4. Statistical analysis

We compared baseline characteristics of patients who developed a severe infection during follow-up with patients who did not, using standard descriptive statistics. The incidence of severe infections was calculated as events per 100 patient-years. Time-to-infection was analysed using a Cox proportional hazard model. Some patients developed multiple severe infections; to adjust for multiple infections within the same individuals, we analysed time-to-infection using a Cox proportional hazard model modified for the multiple failures (infections) per subject data (Anderson-Gill model). Crude time-to-infection was displayed using Kaplan-Meier survival curves. The relationship between severe (or any) infection and a variety of potential predictors (age, sex, diagnosis, disease duration, comorbidities, mean cumulative RTX dose, at-baseline and at-infection glucocorticoid use and dose, concomitant immunosuppressive therapy, such as methotrexate, leflunomide, azathioprine, cyclosporine A, tacrolimus, cyclophosphamide, mycophenolate mofetil, and serum laboratory values, such as neutrophil, lymphocyte, and B-cell counts, IgG, IgA, IgM, and creatinine levels) was investigated. All results are expressed as adjusted hazard ratios (HR) with 95% confidence intervals (CI). To analyse the independent associations between our exposures of interest and incident infections, we performed a multivariate Cox proportional hazard model, adjusting for potential confounders, such as diabetes and other chronic diseases, AID other than RA, concomitant glucocorticoids use, concomitant immunosuppressive therapy, age and sex. The impact of laboratory parameters on RTX safety may vary between disease indications. We decided to pool data of patients treated with RTX in the different indications only if there was no effect modification (interaction) by diagnosis. All P-values < 0.05 (two-sided) were judged significant. Statistical analysis was performed using STATA® Version 12.0 software (StataCorp LP, College Station, TX).

2.5. Funding source

This project was partially funded by an unrestricted research grant from Roche.

3. Results

3.1. Patient characteristics

A total of 168 patients were eligible for enrolment; three refused to participate and four were excluded because of missing data. The remaining 161 patients accounted for 722 RTX infusions. Eighty-six patients (53%) had RA and the remaining 75 (47%) had other AID, mainly connective tissue disorders and vasculitis. Mean follow-up was 2.4 years (median 2.0; interquartile range [IQR] 0.90–3.4). Patients' baseline characteristics are detailed in Table 1.

Four (12%) patients who subsequently presented a severe infection were diabetic, compared to 9 (7%) patients without severe infection ($P = 0.339$). Other chronic co-morbidities predisposing to infections (malignancy treated by chemotherapy and/or radiotherapy, heart failure, chronic kidney failure, nephrotic syndrome, liver failure, splenectomy, HIV infection, primary immunodeficiency,