Pharmacodynamics of rituximab in paediatric immune mediated diseases: B cell depletion and repopulation, effects on immunoglobulin levels and risk for infections

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Abstract

Objective

Rituximab (RTX), used for treatment in paediatric immune-mediated diseases, can lead to hypogammaglobulinaemia and thus to an increased risk of infection, but data on these adverse effects in children are scarce. We aimed to describe the pharmacodynamics of RTX by time to B cell repopulation in paediatric immune-mediated diseases and to assess whether low post-RTX immunoglobulin levels were associated with frequency and severity of infections.

Methods

Data of children with autoimmune diseases (AID), immune dysregulation (ID), haematological diseases (HD) and renal diseases (RD), including immunoglobulin levels pre-/post-RTX and occurrence of infections, who had received RTX at our centre were retrospectively collected. B cell depletion was defined as B cells <10 cells/µl.

Results

Post-RTX B cell depletion was achieved in 45/49 patients. In 30/45 patients with B cell repopulation, median time to repopulation was 166 days (IQR 140–224): AID group (n=9) (183 days (IQR 156–239), ID group (n=6) 170 days (IQR 128–184), HD group (n=7) 139 days (IQR 127–294), RD group (n=7) 160 days (IQR 121–367). Severe infections leading to hospitalisation occurred in 7/52 (13.5%) patients: ID (n=3), HD (n=1), RD (n=3). After RTX treatment, 13/52 patients (25%) had low IgG levels for their age at least once, 11/13 had an infection during low IgG but only 2/13 had a severe infection. Low IgG was not associated with severe infection (p=0.459).

Conclusion

Time to B cell repopulation post-RTX ranged individually but did not significantly differ between paediatric patient groups. Severe infections were non-frequent and not associated with low (post-RTX) IgG levels.

Key words

rituximab, children, B cell depletion, hypogammaglobulinaemia, infections

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Introduction

Rituximab (RTX), a drug leading to B cell depletion, is increasingly used in children for the treatment of autoimmune diseases (AID), immune dysregulation (ID), haematological diseases (HD) and renal diseases (RD) (1-4). RTX is a chimeric anti-CD20 monoclonal antibody targeting CD20 positive B cells (B lymphocytes) that acts as an immunosuppressive agent. Replenishment of B cells by RTX-resistant B cell precursors and continuous production of protecting antibodies by plasma cells render RTX a relatively safe immunosuppressant (5). Therapeutic effect has been suggested to depend on effective depletion of B cells, leading to less production of pathological auto-antibodies (5-7). In patients with rheumatoid arthritis (RA), complete B cell depletion is regularly seen (5, 6). In other autoimmune diseases, such as systemic lupus erythematosus (SLE) and ANCA-associated vasculitis, incomplete B cell depletion has been reported in various adult patients (6, 7). Differences between patient groups in achieving complete *versus* incomplete B cell depletion can be related to presence of anti-drug antibodies (ADA) with possibly higher risk for ADA in patients with SLE (8).

Time to repopulation of B cells after the use of RTX also varies between adult patients with RA, vasculitis and connective tissue diseases (CTD) (6). Knowledge about the time to repopulation is important in terms of planning next dosages, which are commonly given every 6 months. Studies on the duration of B cell depletion in children are scarce and studied populations are small. In children with nephrotic syndrome or SLE, a median duration of 3–4 months until B cell repopulation has been described, with a maximum of 9 months (9) and 12 months (10), respectively. One study in children with SLE described a median of 8 months (11). A study in children with autoimmune central nervous system (CNS) diseases observed B cell depletion for more than 12 months (12). Dosage regimens in these studies varied from 188 mg/m^2 body surface area to 1500 mg/m^2 per course, with the most commonly used regimens comprising 2–4 weekly doses or 2 doses 2 weeks apart (with doses ranging from 375 mg/m^2 to 500 mg/m^2).

RTX-treatment in adults has been associated with an increased risk of infection and several studies have shown that patients with post-RTX hypogammaglobulinaemia develop more serious infectious episodes compared to patients without hypogammaglobulinaemia (13-15). Other studies in adults have shown no relation between low immunoglobulin (Ig) levels after RTX and the occurrence of infections (16, 17). Furthermore, it has been suggested that occurrence of post-RTX hypogammaglobulinaemia is related to the underlying disease. A decline in IgG, IgA and IgM is seen in adult patients with ANCA-associated vasculitis after RTX (6, 14, 15), but not in patients with RA or connective tissue disease like SLE (6). The risk of infectious complications following RTX treatment also seems to differ between patient groups. Higher rates of infections are reported in adult patients with renal diseases (21.6 per 100 patient years) (18) and SLE (6.6–16.6 per 100 patient years) (19, 20) compared to patients with RA (1.9–5.6 per 100 patient years) (21, 22). After kidney transplantation, patients receiving RTX have the highest rate of infections, with an incidence of approximately 50% (23, 24). A higher risk of infections might also be associated with the concomitant use of multiple immunosuppressants. The infections described in adult post-RTX studies were bacterial (pneumonia, cellulitis, clostridium difficile colitis, sinusitis, sepsis, reactivation of tuberculosis), viral (cytomegalovirus, herpes simplex virus, varicella-zoster virus, viral tracheitis, reactivation of hepatitis B virus) and of fungal origin (candidiasis, pulmonary aspergillosis, pneumocystis jirovecii pneumonia, pityriasis versicolor) (14, 18, 25-29).

The pharmacodynamics of RTX on the immune system in adults has been well studied (6, 14, 18, 19, 25, 30), whereas less is known about its effects on the immune system in children (30). It is important to study the paediatric population separately as their immune system is still developing, with possible different effects of RTX. Scarce

data on infection incidence in children treated with RTX assume a relationship with low immunoglobulin, but this is not well established (11, 12, 31, 32). Our objective in this study was to investigate the time to B cell repopulation after RTX treatment in different paediatric patient populations and to investigate the incidence of severe infections in patients treated with RTX in relation to post-RTX IgG levels.

Methods

Patients and data collection

This was a retrospective cohort study in children treated with RTX at the Amsterdam University Medical Centers (Amsterdam UMC), a tertiary centre, between March 2012 and March 2022. Patients were included if they had received their first RTX treatment under the age of 18 years. Patient data were collected from the electronic health record. Collected data were processed anonymously and coded. Studied patients were assigned to different patient groups: autoimmune diseases (AID), immune dysregulation (ID), haematological diseases (HD) and renal diseases (RD). The Medical Research Involving Human Subjects Act (Wet Medisch-wetenschappelijk Onderzoek met mensen, WMO) did not apply to this study and official ethics approval of this study was waived by the medical ethics committee.

Data collected from the electronic patient database included type of disease, date of birth, sex, body height and weight, number/dates and doses of RTX infusions, B cell measurements pre-/post-RTX, IgG-levels pre-/post-RTX, antimicrobial treatment(s) with dates, onset and agent of infections, severity of infections, immunosuppressive use adjuvant to RTX (co-medication), intravenous immunoglobulin (IVIG) therapy, plasmapheresis and stem cell transplantation (SCT). In our hospital according to a clinical protocol IgG levels and B cells were measured pre- and post-RTX 7–14 days after first infusion of the cycle and every 3 months thereafter. IgG levels, onset of infections and IVIG therapies were noted until two years after the last RTX treatment which was defined as the study period. Absolute numbers of B cells were determined with flowcytometry using Multitest 6-colour reagents (BD Biosciences, San Jose, Calif) according to the manufacturer's instructions. Dates of B cell measurements were used to determine time from last treatment to repopulation of B cells. Dosage per RTX infusion was calculated in mg/m^2.

Primary outcome

The primary outcome for this study was the median time to B cell repopulation after RTX in the different patient groups. The definition of time to B cell repopulation was the time between the last RTX dose and the first detection of B cell repopulation. B cell repopulation was defined as a value above the cut-off value for B cell depletion (<10 cells/μl).

Secondary outcomes

Secondary outcome measures were the pre-/post-RTX immunoglobulin (IgG) levels and the occurrence and type of infections during RTX treatment until two years after last infusion. IgG levels of patients who underwent plasmapheresis and/or were treated with IVIG were excluded for this sub-analysis as these treatments lead to removal, respectively replenishment of IgG levels, making it impossible to determine the effect of RTX on patient's IgG levels. The cut-off period for a possible effect of IVIG on the IgG level was set at three months. Because of heterogeneity in duration of RTX treatment we divided patients by the number of RTX infusions for the analyses of IgG levels. IgG levels during and after RTX treatment were classified by quarter, *i.e.* IgG (0–3 m) represents the (lowest) IgG level measured within the first 3 months after treatment, IgG (3–6m) the (lowest) IgG level measured after 3–6 months etc. IgG (>24m) represents (lowest) IgG levels measured from 2 years after first treatment. Post treatment IgG levels were compared with levels before first RTX dose (IgG0). IgG levels were classified as 'low' when below the minimum reference value or as 'normal' when within the reference value for the patient's age (33). Infections were categorised as either mild, moderate or severe. Mild infections included viral upper respiratory tract infections, gastroenteritis and influenza. Moderate infections were bacterial infections requiring oral antibiotic treatment, and severe infections were infections requiring hospital admission.

Statistical analysis

Patient characteristics are presented as medians and interquartile ranges for continuous variables and counts and percentages for categorical variables. The Kruskal-Wallis test was used to compare median time to B cell repopulation between patient groups. Logistic regression was used to estimate the relationship between cumulative doses of RTX and occurrence of low IgG and for the relationship between low IgG levels and the occurrence of total infections and severe infections. Paired samples t-test was used to compare the IgG medians before and after RTX treatment. The chi-square test was used in comparing the occurrence of infections between patient groups. If assumptions for chi-square were not met, the *p*-value of the Fisher's exact test was noted. In all tests, *p*-values were considered significant if the two-sided *p*-value was <0.05. In case of logistics regression analysis, the odds ratio (OR) and 95% confidence interval (CI) were calculated using Binary Logistic Regression. The data were analysed using SPSS Statistics for Windows v. 26.0 (IBM Corp. Armonk, NY).

Results

Patients' characteristics are summarised in Table I. In total, the study population consisted of 52 patients. The AID group included 23 children with the following diagnoses: SLE (n=10), ANCA-associated vasculitis (n=3), neuromyelitis optica spectrum disease (NMOSD) associated with anti-AQP4 or anti-MOG antibodies (n=3), rheumatoid factor positive juvenile idiopathic arthritis (JIA) (n=2), juvenile systemic sclerosis (n=2), juvenile dermatomyositis (JDM) (n=1), myasthenia gravis (MG) (n=1) and multifocal motor neuropathy (MMN) associated with GalC antibodies (n=1). The group with ID included 9 patients: haemophagocytic lymphohistiocytosis (HLH) (n=3),

Table I. Patients' characteristics.

Patients	Total		Autoimmune disease		Immune dysregulation		Haematological disease		Renal disease	
Patients, n (%)	52		23 (44)		9 (17)		7 (13)		13 (25)	
Female/male, n	28/24		16/7		4/5		3/4		5/8	
Age in years at RTX1, median (IQR)	12.9 (8.3-15.8)		13.7 (12.2-17)		9.4 (2.7-13.7)		10 (7.5-14.1)		10.1 (5.1-15.7)	
Number of total infusions RTX, median (IQR)	3 (2-6)		4 (2-6)		3 (2-8)		3 (2-6)		2 (1.5-3)	
Cumulative dose RTX (mg/m^2), median (IQR)	1133 (750-2306)		1574 (750-3955)		1150 (750-3625)		1372,5 (900-2250)		1125 (605-1133)	
Co-medication, n (%)	During	After	During	After	During	After	During	After	During	After
- Corticosteroids only	13 (25.0)	10 (19.2)	5 (21.7)	2 (8.7)	2 (22.2)	3 (33.3)	3 (42.9)	2 (28.6)	3 (23.1)	3 (23.1)
- Other immunosuppressant(s)	3 (5.8)	5 (9.6)	1 (4.3)	3 (13.0)	0	1 (11.1)	1 (14.2)	1 (14.2)	1 (7.7)	0
- Combination*	30 (57.7)	34 (65.4)	17 (73.9)	18 (78.3)	4 (44.4)	5 (55.5)	1 (14.2)	2 (28.6)	8 (61.5)	9 (69.2)
- Cytostatic agent	2 (3.8)	3 (5.8)	1 (4.3)	2 (8.7)	1 (11.1)	1 (11.1)	0	0	0	0
- No immunomodulatory co-medication	4 (7.7)	3 (5.8)	0	0	2 (22.2)	0	1 (14.2)	2 (28.6)	1 (7.7)	1 (7.7)
- IVIG	7 (13)	11 (21.2)	4 (17.4)	5 (21.7)	0	3 (33.3)	1 (14.2)	2 (28.6)	2 (15.4)	1 (7.7)

*Steroid plus other immunosuppressant(s) incl cytostatic. [1]During first RTX cycle, [2]After first cycle, during follow-up.
5 patients underwent plasmapheresis in the studied period.

idiopathic pulmonary haemosiderosis (IPH) (n=2), MALT lymphoma in Sjögren disease (n=1), ataxia-telangiectasia (n=1), X-Linked lymphoproliferative disease type 1 (n=1), Graft versus host disease (GvHD) after allogenic stem cell transplantation (n=1). The third group consisted of 7 patients with HD with the following diagnoses: immune thrombocytopenia (ITP) (n=5), autoimmune haemolytic anaemia (AIHA) (n=2). The fourth group concerned 13 patients with RD; nephrotic syndrome (n=8), renal transplantation with rejection (n=4), membranoproliferative glomerulonephritis (n=1).

The median age at the first RTX treatment (RTX1) was 12.9 years (IQR 8.3–15.8). The median number of RTX infusions received was 3 (IQR 2–6). Most patients received co-medication during RTX treatment (49/52, 94.2%). Concomitant immunosuppressants other than steroids were methotrexate, tacrolimus, sirolimus, mycophenolic acid, azathioprine, hydroxychloroquine, leflunomide, cyclosporin, cyclophosphamide and infliximab.

B cell repopulation

The number of B cells was not tested in three patients. In accordance with the clinical protocol, most patients (n=44/49) were tested for B cell depletion after the first RTX dose of a cycle (median 11 days (IQR: 7–14)). In the remaining 5 patients B cell number was determined later in the treatment course. Post-RTX B cell depletion was achieved in 45/49 (91.8%) patients. B cell depletion after one RTX dose was achieved in 40/44 (90.9%) patients. In 4/49 (8.2%) patients B cell depletion was not achieved. These were all patients with an AID (3 with SLE and 1 with NMOSD).

In the B cell depleted patients, B cell repopulation was measured in 66.7% (30/45). Median time until detection of repopulation was 166 days (IQR 140–224). No statistical differences were found for time to B cell repopulation between patient groups (AID group 183 days (IQR 156–239), ID group 170 days (IQR 128–184), HD group 139 days (IQR 127–294), RD group 160 days (IQR 121–367) (p=0.184)). In twenty patients (20/30, 66.7%) B cell repopulation was observed within 6 months after RTX treatment. In two patients repopulation was observed after more than a year (367–444 days). Both patients had RD (nephrotic syndrome).

IgG levels

Seven patients were excluded for this sub analysis. These patients had been treated with plasmapheresis or IVIG in the previous 3 months, one patient received a haematopoietic stem cell transplantation (HSCT) and had low IgG level after transplantation. Median IgG0 level was 8.9 g/L (IQR: 6.0–11.2 g/L). In 33/45 patients (73.3%), pre-RTX IgG levels were within the normal range for their age (median 9.0 g/L, IQR: 6.6–11.0, range 4.9–18.4 g/L) (33). In 7 patients (15.5%) pre-RTX IgG levels were low for their age (median 4.6 g/L, IQR: 4.3–4.7, range 3.8–5.1 g/L). Median IgG (3–6m) level was 8.4 g/L (IQR: 5.1–10.6 g/L) and median IgG (6-9m) was 8.5 g/L (IQR: 4.9–11.9 g/L). The median follow-up time for IgG was 18 months (IQR: 12–18 months).

Figure 1A shows the course of the IgG levels at different time points post-RTX in the 22 children who had received one cycle of RTX (1 or two doses). Twelve children had received 3 or 4 doses of RTX (Fig. 1B), 8 children had received 5 or 6 doses of RTX (Fig. 1C) and 8 children had received 7 doses or more (up to 20) (Fig. 1D).

IgG levels varied over time during RTX treatment. However, median IgG levels at all time points were not statistically different from IgG0. There was also no association between cumulative doses of RTX (mg/m^2) and the occurrence of low IgG levels (OR=1.000, 95% C.I: 0.999–1.000, p=0.388). Long-lasting hypogammaglobulinaemia (low IgG during 6 months after the last RTX dose) occurred in 4 (7.7%) cases, of which 2 (3.8%) had persisting hypogammaglobulinaemia for 3 years after the last RTX dose.

Onset of infections

Thirty-six patients (36/52, 69.2%) developed an infection (of any kind) within two years after the last RTX dose, with an infection rate of 0.79

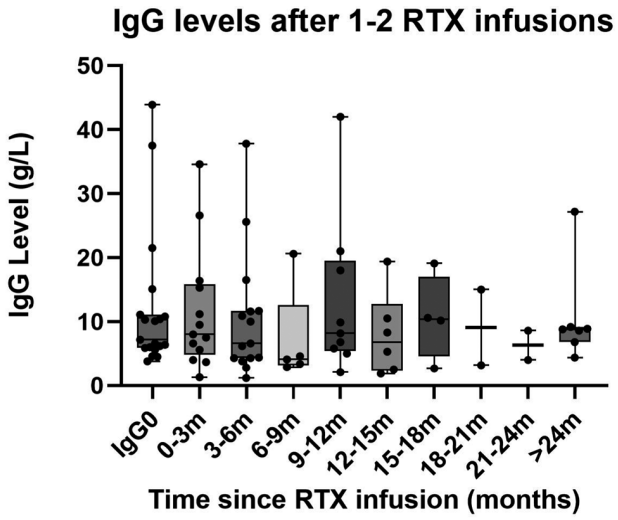

Fig. 1A. IgG levels in children receiving 1-2 RTX infusions.

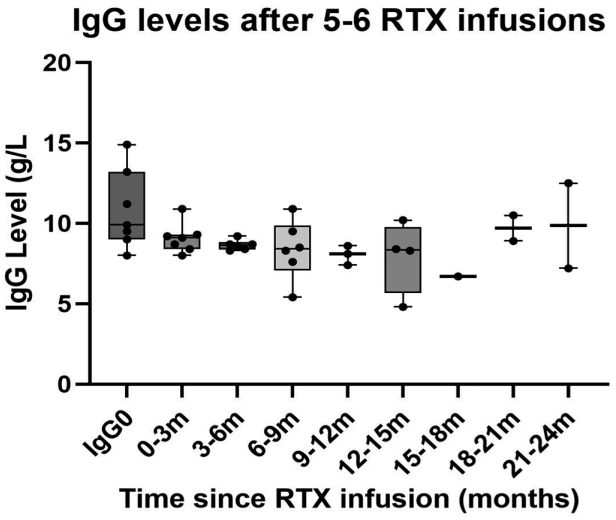

Fig. 1C. IgG levels in children receiving 5-6 RTX infusions.

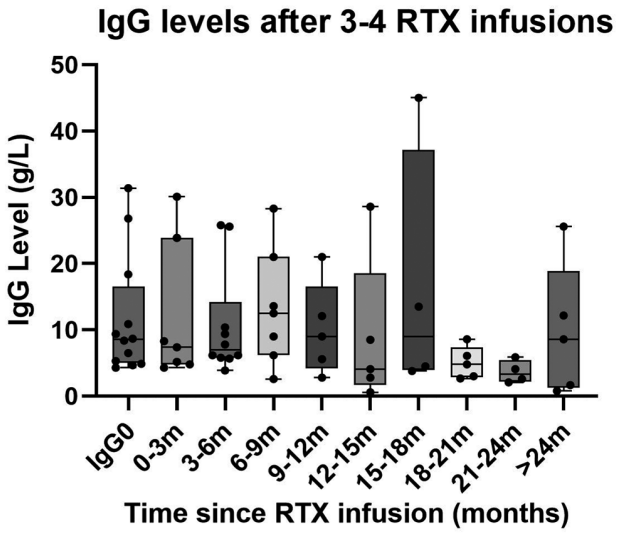

Fig. 1B. IgG levels in children receiving 3-4 RTX infusions.

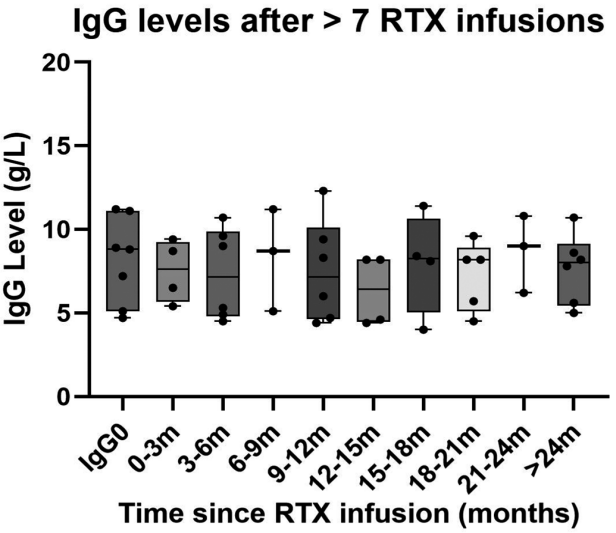

Fig. 1D. IgG levels in children receiving >7 RTX infusions.

per person-year. Infections during plasmapheresis (n=1) and shortly after SCT (n=1) were excluded. There was no significant difference in occurrence of infections between patient groups (p=0.278). The number of patients with infections per group, is displayed in Figure 2. Seven patients (13.5%) had in total 11 severe infections (one patient was admitted 3 times and three patients were admitted twice), with a severe infection rate of 0.11 per person-year. Diagnoses included viral upper airway infection (n=3), pneumonia (n=4), fever of unknown cause (n=1), gastro-enteritis (n=1), infected duplication cyst (n=1), appendicitis (n=1). Two of these patients were on antibiotic prophylaxis (cotrimoxazole or azithromycin), while two were started on antibiotic prophylaxis after treatment of a severe infection.

Pathogens that were isolated were viruses (respiratory syncytial virus (RSV), SARS-CoV-2, cytomegalovirus (CMV), adenovirus, rhinovirus, human bocavirus (HboV), rotavirus, astrovirus, norovirus) and bacteria (Staphylococcus aureus, Staphylococcus epidermis, Salmonella type C, Pseudomonas aeruginosa and Klebsiella pneumoniae). Microbiological tests were not requested by default during an infection, but only if there was a clinical indication.

After RTX treatment 13/47 patients (27.7%) had low IgG levels on at least one occasion of whom 11 (23.4%) had an infection during low IgG levels. There was no statistic significant association between low IgG and the occurrence of an infection (OR 3.348, 95% C.I: 0.645–17.373, p=0.150) or the occurrence of a severe infection (OR 2.061 95%, C.I: 0.304–13.971, p=0.459). Of the 7 patients with a severe infection, 5 had no hypogammaglobulinaemia.

Discussion

In this study, we describe time to B cell depletion following treatment with the CD20 monoclonal antibody RTX, time to B cell repopulation post-treatment, pre- and post-RTX IgG levels and infections in 52 children. The combination of all these post-RTX data, de-

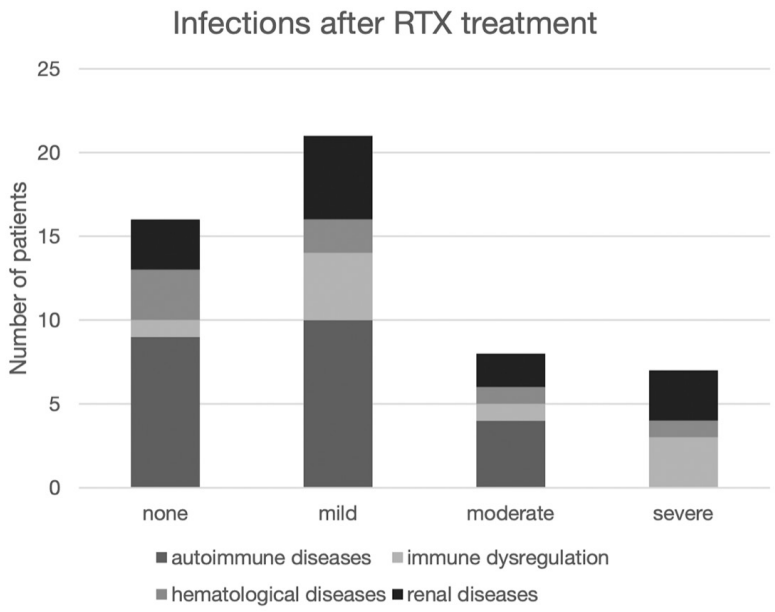

Fig. 2. Number of patients per type of infection.

scribing RTX pharmacodynamics in a paediatric cohort, is the first study of its kind.

Post-RTX B cell depletion was achieved in the majority of patients. Of the 4 patients that failed B cell depletion, 3 were SLE patients in whom ADA against RTX were detected, implying a relationship between ADA and incomplete B cell depletion in these patients. ADA are known to have the capability to neutralise the effect of RTX on B cells and influence clinical outcome (8, 34). ADA were however not tested in all patients, as this was outside the scope of this study. Another patient without B cell depletion was a patient with NMOSD. This patients' B cells did decline, but were just above the set threshold of 10 cells/μl. There is a possibility that this patient also had ADA, but they were not tested. Furthermore, it is also possible that this patient did achieve B cell depletion later, but it was not measured. Time to repopulation did not significantly differ between our patient groups. Median time until repopulation was 166 days (IQR 140–224). This is in line with the scarce studies done before: in children with nephrotic syndrome or SLE, a median duration of 3–8 months until B cell repopulation has been described (9-11). In two patients, repopulation was achieved after more than a year (367–444 days). Both of these patients had RD (nephrotic syndrome). Russell *et al*. also observed B cell depletion for more than 12 months in children with autoimmune central nervous system (CNS) diseases (12), although they also reported that this could be an underestimation as serial measurement was not routinely performed. In our patients, measurements were done routinely and there was a definite long-lasting depletion of B cells after one cycle of RTX (2 doses). Concomitant immunosuppressants such as steroids and DMARDs were used in most patients. Only 4 patients did not receive any co-medication at all. Some patients received only steroids, but most patients received a combination of steroids and other immunosuppressants. Frequency and dosages of these drugs varied. The immune-modulating effect of these drugs could possibly have influenced our outcomes, but due to the heterogeneous co-medication regimens it was not possible to draw reliable conclusions on the relationship between different adjuvant immunosuppressive medication use and duration of B cell depletion. When comparing studies, it is important to realise that different definitions for cut-off points of (CD19+) B cell depletion and repopulation are used. Our definition of B cell repopulation was a value above the cut-off value of peripheral B cell depletion (10 cells/μl) frequently used in studies (35-37). Although percentages are also being used in clinical practice, most studies use absolute values instead of percentages to enable comparison. The LUNAR study used a definition of CD19 count <20 cells/μl (20) for peripheral B cell depletion, in the phase I trials of rituximab in SLE a CD19 count <5 cells/μl was used as a cutoff (38) and others defined complete peripheral depletion as a CD19 count of 0 cells/μl (39, 40). However, there is no absolute definition available.

Severe infections leading to hospitalisation occurred in only 7 (13.5%) patients (with 11 infections). This infection rate is remarkably low, as overall incidence of infections in paediatric auto-immune diseases varies from 14% up to 57.4% (41-43). Several studies have shown that patients with post-RTX hypogammaglobulinaemia develop more serious infections compared to patients without hypogammaglobulinaemia (13-15). However, other studies have found no such relationship (16, 17). In our cohort, we found no association between the occurrence of infections and low post-RTX IgG levels. In the 7 cases with a severe infection, only 2 patients (28.6%) had low post-RTX IgG levels for their age. We defined a severe infection as an infection leading to hospitalisation, which is a common definition used. One could discuss this definition, as the threshold of admission of patients treated with RTX is probably lower beforehand. For instance, infections in this category also included viral upper airway infections, viral gastro-enteritis and fever without cause in our cohort. One patient developed a mild viral infection which led to exacerbation of her disease. She was admitted in the hospital because of the exacerbation and not because of severity of the infection. Infections during plasmapheresis and after SCT were excluded. One patient developed a COVID-19 pneumonia and was admitted to the Paediatric Intensive Care Unit (PICU) during plasmapheresis treatment for an AID. Another patient developed a severe colitis, but this was in the period after a SCT. Fourteen patients were on antibiotic prophylaxis during (a part of) the study period, pre-

sumably reducing the number of infections. This would mean that the number of post-treatment infections may have been underestimated.

Because of the relatively small number of patients in paediatrics, we decided to include all children treated with RTX at our paediatric department and to not limit to children with specific diseases, making our cohort heterogeneous. We did cluster the patients into several subgroups based on the underlying pathophysiology and subsequently performed sub-analyses on those patient groups. We did not find any statistical differences in occurrence of infections between our different patient groups. Some studies argue that post-RTX hypogammaglobulinaemia and the risk of infectious complications after RTX treatment depends on the type of disease. Patients receiving RTX after kidney transplantation, are known to have very high infection rates, with an incidence of approximately 50% (23, 24). In these cohorts, more bacterial and fungal infections are seen and this is ascribed to the T-cell depleting agents used in these patients for pretransplant immunosuppressive induction or for anti-rejection therapy. In our cohort, the underlying conditions of patients with severe infections varied: renal transplantation with rejection, nephrotic syndrome (n=2), GvHD after allogenic stem cell transplantation, IPH, X-linked lymphoproliferative disease and AIHA.

Pre-RTX IgG levels were low in 7 patients, of which 5 were from the RD group. These patients had severe proteinuria, explaining these lower pre-RTX IgG levels. Five patients (11.1%) had IgG levels above normal range, 4 of them were SLE patients. Elevated IgG is often seen in SLE, consistent with the increased immune activity in this systemic disease. Long-lasting post-RTX hypogammaglobulinaemia (>6 months) occurred in 4 (7.7%) cases. Two patients were from the AID group (juvenile systemic sclerosis and NMOSD) and returned to normal IgG levels within a year. One patient received antibiotics twice (upper airway infection) and the other one did not have any infectious complications. One patient with persisting hypogammaglobulinaemia (>3 years) was a patient with nephrotic syndrome and was admitted once for antibiotic treatment because of fever. The other one, a patient with GPA, just had two viral upper airway infections during follow-up. Both patients were started on co-trimoxazole prophylaxis after their first infection.

A limitation of our study is the retrospective data with differences in timing of measurements during follow-up. The blood samples for B cell detection and IgG levels were not always taken routinely after RTX infusions but details could be descriptively specified in our results. A strength of our study is the low probability of missing infections due to the accessibility to healthcare and shared-care constructions in The Netherlands.

In our patients there was no difference in timing of B cell repopulation between different patient categories but there were individual differences. By measuring B cell repopulation after RTX treatment in individual paediatric patients, this may lead to adjusted personalised therapy approaches, in terms of timing of new RTX cycles to make it a more efficient treatment. In our cohort, there was no correlation between low IgG and infections but (severe) infections did occur. We advise to measure IgG concentrations after RTX treatment every 3 months and consider antibiotic prophylaxis in those with hypogammaglobinaemia as this protective effect against infections was not studied.

Conclusion

Time to B cell repopulation in children ranged individually but did not significantly differ between patient groups. Measuring B cell repopulation may be helpful in personalised treatment approaches, by individualising RTX infusion schedules. Low post-RTX IgG levels were only seen in a minority of paediatric patients after RTX treatment. Low post-RTX IgG levels were not associated with (severe) infections, however we do advise to consider antibiotic prophylaxis in those with hypogammaglobinaemia. This study indicates that RTX seems a relatively safe treatment in paediatric immune mediated diseases with some precautions in mind.

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