B cell subsets reconstitution and immunoglobulin levels in children and adolescents with B non-Hodgkin lymphoma after treatment with single anti CD20 agent dose included in chemotherapeutic protocols: single center experience and review of the literature

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Background. RTX, an anti-CD20 monoclonal antibody, added to chemotherapy has proven to be effective in children and adolescents with high-grade, high-risk and matured non-Hodgkin lymphoma. RTX leads to prompt CD19+ B lymphocyte depletion. However, despite preserved immunoglobulin production by long-lived plasmablasts after treatment, patients remain at risk of prolonged hypogammaglobulinemia. Further, there are few general guidelines for immunology laboratories and clinical feature monitoring after B cell-targeted therapies. The aim of this paper is to describe B cell reconstitution and immunoglobulin levels after pediatric B-NHL protocols, that included a single RTX dose and to review the literature.

Methods. A retrospective single-center study on the impact of a single RTX dose included in a chemotherapeutic pediatric B Non-Hodgkin Lymphoma (B-NHL) treatment protocols. Immunology laboratory and clinical features were evaluated over an eight hundred days follow-up (FU) period, after completing B-NHL treatment.

Results. Nineteen patients (fifteen Burkitt lymphoma, three Diffuse large B cell lymphoma, and one Marginal zone B cell lymphoma) fulfilled the inclusion criteria. Initiation of B cell subset reconstitution occurred a median of three months after B-NHL treatment. Naïve and transitional B cells declined over the FU in contrast to the marginal zone and the switched memory B cell increase. The percentage of patients with IgG, IgA, and IgM hypogammaglobulinemia declined consistently over the FU. Prolonged IgG hypogammaglobulinemia was detectable in 9%, IgM in 13%, and IgA in 25%. All revaccinated patients responded to protein-based vaccines by specific IgG antibody production increase. Following antibiotic prophylaxes, none of the patients with hypogammaglobulinemia manifested with either a severe or opportunistic infection course.

Conclusion. The addition of a single RTX dose to the chemotherapeutic treatment protocols was not shown to increase the risk of developing secondary antibody deficiency in B-NHL pediatric patients. Observed prolonged hypogammaglobulinemia remained clinically silent. However interdisciplinary agreement on regular long-term immunology FU after anti-CD20 agent treatment is required.

Key words: rituximab, B non-Hodgkin lymphoma, chemotherapy, late complications of chemotherapy, hypogammaglobulinemia, children and adolescents

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INTRODUCTION

Non-Hodgkin lymphomas (NHL), a heterogeneous group of lymphoid malignancies, is the third most common malignancy in children, accounting for seven percent of pediatric cancer in general[1-4]. Adding rituximab (RTX) to chemotherapeutic treatment protocols has proven to be effective in children and adolescents with high-grade, high-risk, mature B non-Hodgkin lymphoma (B-NHL) leading to long-term complete remission in 95% (ref.[3]) of patients[3,5-8].

However, the introduction of RTX into the B-NHL treatment protocols is followed by the risk of development of secondary antibody deficiency and infections[3,9-11]. It also remains true that the pediatric data on long-term B cell reconstitution and antibody production after RTX in treatment protocols in B-NHL patients are inconsistent[3,12].

RTX, a chimeric, genetically engineered, murine/human monoclonal antibody (mAb) directed against the membrane-embedded cluster of differentiation (CD) 20 antigen, is a B cell-targeted therapy (BCTT) (ref.[13,14,15-17]). It causes rapid complete transient depletion of CD20 positive B cell subsets[9]. However, RTX spares premature pro-B cells and long-lived plasmablasts (LLP) which lack CD20 expression[9,17-23]. Its clinical efficacy depends on the

dosage, the number of administrations, its diffusion into the tissues and elimination kinetics[24].

Secondary antibody deficiency is widely reported as 10% to 40% (ref.[9,14,15,25-27]) in adults treated with RTX for autoimmune diseases and malignancies[9,14,15,25-27]. The impaired or failed B-cell reconstitution after the B lymphocyte targeted treatment together with altered antibody production sporadically requires introduction of an immunoglobulin replacement therapy (IGRT) in the most severely affected patients[28,25,29-32]. Rarely, B-targeted treatment of malignancies unmasks underlying inborn error of immunity (IEI) that may be mistaken for secondary immunodeficiency[33,34].

To date however, there is a limited body of evidence that the pediatric chemotherapeutic protocols commonly used before the introduction of antiCD20 mAb into pediatric B-NHL treatment, promote development of secondary immunodeficiency, including hypogammaglobulinemia after the end of B-NHL treatment. Published reports have concentrated predominantly on patients suffering from acute lymphoblastic leukemia, HIV or acquired immunodeficiency syndrome and inborn errors of immunity[33-37]. In addition, the list of chronic health conditions and long-term late complications of childhood, adolescent and young adult NHL survivors does not specificaly include secondary immunodeficiencies[38-40]. Evidence on the contribution of individual agents included in B-NHL chemotherapeutic protocols (cytarabine, etoposide, cyclophosphamide, isophosphamide, daunorubicin, methotrexate, prednisolone, dexamethasone), to the development of secondary antibody deficiency after the B-NHL treatment is limited[28,41-44].

RTX concomitant therapy with purine analogs or mycophenolate mophetil may co-orchestrate hypogammaglobulinemia, whereas the synergic effect of RTX and cyclophosphamide or corticosteroids on immunoglobulin values remains controversial[9,30,45,46].

This retrospective study aimed to evaluate the clinical and laboratory parameters during the first eight hundred follow-up days after the end of treatment in pediatric B-NHL, where a single anti CD20 agent dose was administered during the B-NHL treatment course.

MATERIALS AND METHODS

Eligibility

A retrospective analysis of the impact of B-NHL chemotherapeutic protocols that included a single rituximab dose, on clinical and laboratory immunology features was conducted. The analyses focused on serum immunoglobulin levels and CD19+ B lymphocyte subsets in pediatric and adolescent B-NHL patients over the first 800 days of the follow-up (FU) period after the B-NHL end-of-treatment (EoT) visit. The research project was approved by Brno University Hospital Ethics Committee.

Patients under nineteen years of age with diagnosed *de novo* mature B-NHL classified by the Revised European-American Lymphoma (REAL) criteria were eligible, including patients with St. Jude Stages III/IV (ref.[47-49]). Patients with a known acquired immunodeficiency before the B-NHL diagnosis, those with an inborn error of immunity and those with organ transplant prior to B-NHL diagnosis were excluded. Patients treated with checkpoint inhibitors, anti CD19+B cell targeted therapies, apart from a single RTX dose, and patients treated with multiple anti CD20 agent doses including RTX were also excluded.

Hypogammaglobulinemia was defined as an IgG, IgM, and IgA decrease under the lower range of age-adjusted reference values for each age group.

The time of B lymphocyte subset reconstitution initiation was defined as the day of B cell subset evaluation when 1% of B cells were detectable in the blood after the CD19+B cell depletion period (CD19+ ≥ 1%).

Severe infection was defined as febrile bacterial infection or disseminated fungal or herpetic infection necessitating hospital admission and parenteral (antibiotics, antimycotics, or virostatics) treatment.

Antibody deficiency requiring immunoglobulin replacement therapy was defined as IgG hypogammaglobulinemia with impaired response to protein and polysaccharide vaccine antigen[31].

Laboratory settings

B cell characteristics were based on $CD19^{+}$ expression measured and analyzed by multiparameter flow cytometry and flow cytometry software (Navios, Beckman Coulter Inc, Brea, CA, USA). B-cell subsets were identified based on the defined surface marker expression according to the maturation stage and recognized as CD24++CD38++ (transitional B cells), CD27-IgM+ (naïve B cells), CD27+IgM+ (marginal zone-like B cells), CD27+IgM– (isotype switched memory B cells), CD27++CD38++ (plasmablasts) and CD21low CD38low (CD21low B cells). B cell and B cell subsets' age-adjusted reference ranges were based on Morbach et al. data and expressed as a percentage[20,50] .

Quantification of immunoglobulin class levels (IgG, IgA, and IgM) was determined by a commercial nephelometry assay (Immage 800, Beckman Coulter Inc. Brea, CA, USA). Age-adjusted reference ranges of immunoglobulin IgG, IgA, and IgM classes were based on the IMMAGE 800 Immunochemistry Systems, Chemistry References, Beckman Coulter, Inc. values. Immunoglobulin values were expressed in grams per liter (g/L).

Enzyme immunoassay kits (The Binding Site Group Ltd, Birmingham, Great Britain, VaccZyme™ Human Anti-Hemophilus influenzae Type b EIA Kit ref. number: MK 016, VaccZyme™ Human Anti-PCP IgG EIA Kit, ref. number MK 012, VaccZyme™ Human Anti Tetanus Toxoid IgG EIA Kit: MK10) were used to quantify specific antibodies, anti- tetanic toxoid IgG antibodies (anti-TET IgG), anti-Hemophilus infuenzae type B IgG specific antibodies (anti-HiB IgG) and anti-Streptococcus pneumoniae capsular polysaccharide IgG specific antibodies (anti-PCP IgG). Reference range values were provided by the manufacturer, The Binding Site Group Ltd, Birmingham, Great Britain. The anti-PCP and anti-HiB values were expressed in milligrams per liter (mg/L) and

anti-TET antibodies were expressed in international units per milliliter (IU/mL)

Statistical analysis

The eight hundred days follow-up period was divided into four two hundred days follow-up (FU) intervals, to reduce the scarcity of the dataset. For each interval the 1st, 2nd (median) and the 3rd quartile (Q1, Q2 and Q3) were calculated. The interquartile range (IQR), IQR = (Q3–Q1), served as a measure of dispersion (variability). The choice of medians and IQR reflected the nature of the data, were more robust and less susceptible to outliers than e.g, mean and standard deviation.

To overcome the age differences between the subjects (where value difference ranges for immunoglobulins and B cell subsets vary between define age groups) and the limited size of the dataset, the data were standardized by dividing the measured values of immunoglobulin levels and B cell subsets count by the lower bound of age adjusted reference values. The results are displayed in rationalized (dimensionless) units.

In addition, for the three immunoglobulin classes (IgG, IgA, and IgM), a complementary description of temporal dynamics during each FU period was provided by calculating the proportion of patients with hypogammaglobulinemia within each FU interval. For each proportion, the corresponding 95% (Clopper-Pearson) binomial confidence interval (CI) was calculated.

The results are illustrated by the bar graphs, and tables.

RESULTS

Of the twenty-six pediatric and adolescent patients treated for mature B-NHL in a single pediatric oncology university centrum between the years 2011–2019, nineteen patients fulfilled the inclusion criteria (14 male and 5 female).

Fifteen patients suffered from Burkitt lymphoma B-NHL BL (median NHL onset age 8.5 years), three from diffuse large B cell lymphoma B-NHL DLBCL (median NHL onset age 15 years), and one from marginal zone B cell lymphoma (B-NHL onset 16 years). Data are summarized in Table 1.

The treatment was based on B non-Hodgkin lymphoma Berlin-Frankfurt-Münster (B-NHL BFM) 2013 and 2004 treatment chemotherapeutic protocols where a single RTX dose (375 mg/m^2) was included[48].

The median duration of antibiotic prophylaxes (Trimethoprim/Sulfamethoxazole TMP/SMT) after the EoT was 4.5 months. The administered dose was 5 mg/kg of TMP to a maximum of 160 mg TMP component twice a day on two consecutive days per week. One patient presented with an uncomplicated periproctal abscess.

Regarding respiratory tract infections, there were two uncomplicated otitis media and two uncomplicated cases of bronchitis. There were no recurrent sinopulmonary infections over the FU.

None of the patients required immunoglobulin replacement therapy. Because of the lack of laboratory and clinical signs, genetic analysis for IEI was not initiated, none of the patients fulfilled IEI definitions[51-53].

The dynamics of the three immunoglobulin class levels (IgG, IgA, and IgM) over eight hundred days of FU are displayed in Table 2. Descriptive statistics were used to summarize the data on 41, 35, 23, and 15 measurements for IgG (Table 2), 24, 24, 18, and 11 measurements for IgM (Table 2), and 24, 23, 18, and 11 measurements for IgA in I-IV FU intervals (Table 2). The median and IQR for the four FU intervals were set. A consistent increase in all immunoglobulin levels over the FUI is apparent (Table 2).

The proportion of patients with hypogammaglobulinemia for the three immunoglobulin classes (IgG, IgA, and IgM) over the follow-up period is illustrated in Fig. 1 with the corresponding 95% Clopper-Pearson binomial confidence intervals. The results are shown in Table 3. All three immunoglobulin classes consistently increased over the follow-up intervals with diminishing hypogammaglobulinemia proportion/percentage of each immunoglobulin class (Table 3). IgA hypogammaglobulinemia predominated (Table 3).

The B cell reconstitution median was 3 months after EOT. B cell subset medians and corresponding interquartile ranges (IQRs) for the four FU intervals are displayed in Table 4. Descriptive statistics for the FU intervals I–IV were used for 19, 16, 12, and 5 measurements respectively (Table 4).

Table 1. Clinical features of B-NHL patients treated with a single RTX dose included in chemotherapeutic protocols (Eight hundred days of follow-up period after the end of B-NHL treatment).

No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
ID	1	2	3	4	5	6	7	8	9	10	11	12	13	18	22	16	17	19	20
DG	B-NHL BL	B-NHL BL	B-NHL BL	B-NHL BL	B-NHL BL	B-NHL BL	B-NHL BL	B-NHL BL	B-NHL BL	B-NHL BL	B-NHL BL	B-NHL BL	B-NHL BL	B-NHL BL	B-NHL BL	DL BCL	DL BCL	DL BCL	B-NHL
Age	5	11	4	9	13	4	12	10	6	8	9	11	17	8	9	15	7	16	16
Sex	M	M	M	M	M	M	F	M	M	F	M	M	F	F	M	M	M	F	M
Protocol	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013	NHL BFM 04	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013	NHL BFM 2013

ID, Anonymized subject number; Dg; Diagnose – B NHL BL, Non-Hodgkin lymphoma-Burkitt lymphoma; DLBCL, Diffuse large B cell lymphoma; B NHL other, Leukemized marginal zone B cell lymphoma; Age, Age at the time of diagnosis; F, Female; M, Male; Protocol, Non-Hodgkin lymphoma Berlin-Frankfurt-Munster registry 2013 treatment protocol, B Non-Hodgkin lymphoma Berlin-Frankfurt-Munster registry 2004 treatment protocol.

Table 2. The impact of B-NHL chemotherapeutic protocols with a single RTX dose on IgG, IgA, and IgM immunoglobulin levels over a follow-up period of eight hundred days after the end of B-NHL treatment.

Immunoglobulin classes	FU interval (days) 0–200	FU interval (days) 201–400	FU interval (days) 401–600	FU interval (days) 601–800
IgG	0.62 (0.56–0.81)	0.75 (0.46–0.89)	0.90 (0.73–1.11)	0.92 (0.72–1.01)
IgA	0.31 (0.24–0.70)	0.38 (0.29–0.85)	0.47 (0.33–0.57)	0.81 (0.48–1.03)
IgM	0.28 (0.17–0.42)	0.46 (0.31–0.69)	0.44 (0.32–0.59)	0.72 (0.36–0.91)

The median immunoglobulin values are presented in bold, while the first quartile (Q1), and the third quartile (Q3) of immunoglobulin levels in four follow-up intervals are in brackets. Immunoglobulin levels are expressed in rationalized (dimensionless) units.

Table 3. The proportion of subjects with IgG, IgA, IgM hypogammaglobulinemia in the follow-up period of eight hundred days after the end of B-NHL treatment.

FU		IgG			IgA			IgM	
days	Proportion	Lower CI	Upper CI	Proportion	Lower CI	Upper CI	Proportion	Lower CI	Upper CI
0–200	0.36	0.13	0.65	0.67	0.35	0.90	0.67	0.35	0.90
201–400	0.32	0.13	0.57	0.62	0.32	0.86	0.31	0.09	0.61
401–600	0.21	0.05	0.51	0.45	0.17	0.77	0.18	0.02	0.52
601–800	0.09	0.01	0.41	0.25	0.03	0.65	0.13	0.01	0.53

The proportion of subjects with IgG, IgA and IgM hypogammaglobulinemia, along with corresponding 95% confidence intervals for four follow-up intervals, were calculated using the Clopper-Pearson method. The exact binomial confident interval (CI) was used to calculate the proportion. It quantifies uncertainty as a probability of success from a series outcome of success–failure experiments – Bernoulli trials. CI is a range of values, 95% CI expresses the 95 times out of 100 the proportion lies this range.

Table 4. The impact of chemotherapeutic protocols that included a single RTX dose on B-cell subset reconstitution during eight hundred days follow-up after the end of B-NHL treatment.

B-cells subsets	FU intervals (days) Median (Q2) and IQR (Q1–Q3) for B-cell subsets (in rationalized units)			
	0–200	201–400	401–600	601–800
Naïve B cells	1.17 (0.00–1.21)	1.15 (1.10–1.17)	1.07 (1.01–0.59)	1.06 (1.05–1.06)
Marginal Zone B cells	0.24 (0.00–0.42)	0.62 (0.23–0.76)	0.81 (0.59–1.03)	0.89 (0.42–1.32)
Switched Memory B cells	0.16 (0.00–0.33)	0.39 (0.29–0.54)	0.82 (0.44–1.38)	0.61 (0.42–0.64)
Transitional B cells	6.07 (0.00–9.45)	2.93 (2.47–3.70)	2.11 (1.47–3.03)	1.99 (1.18–2.07)
Plasmablasts	0.40 (0.00–0.60)	0.67 (0.40–0.88)	0.76 (0.39–1.46)	0.43 (0.30–0.57)
CD21Low B cells	0.62 (0.00–1.32)	1.00 (0.67–1.09)	1.12 (1.00–1.33)	0.95 (0.80–1.05)

For each FU interval of two hundred days, the median values of B-cell subsets count are presented in bold, while the first quartile (Q1) and the third quartile (Q3) are in brackets. Values are expressed in rationalized (dimensionless) units.

Transitional together with naïve B lymphocyte subsets culminated in the I. FU interval displaying steady decay over the rest of the FU intervals (II–IV.) (Table 4). In contrast, marginal zone B lymphocyte subsets continuously increased over the four FU intervals (I–IV) (Table 4). Switched memory B lymphocyte subsets, plasmablasts, and $CD21^{low}$ B lymphocytes increased over the first three FU intervals (I–III) (Table 4), decreasing again in the last IV (Table 4).

In all nineteen subjects (Table 1), specific IgG antibodies (anti-PCP, anti-TET, and anti-HiB) were evaluated at a median of 4.5 months after the end of B-NHL treatment. A decrease in specific IgG below the lower reference range value was obvious in four subjects in anti-TET (4/19), four subjects in anti-PCP (4/19), and three subjects in anti-HiB (3/19,). Two subjects (2/19) presented with decreased combined anti-PCP and ant-TET specific antibody decrease in specific antibody values.

A decrease under the lower reference range in previously protective specific IgG antibodies was observed in four subjects in anti-PCP (4/19), in one subject in anti-TET (1/19), and in one subject in anti-HiB (1/19).

Protein-based anti-tetanic toxoid vaccine was administered to six subjects (6/19), anti-*Hemophilus influenzae type B* conjugated vaccine was administered to two subjects (2/19). Four subjects (4/19) were revaccinated with a protein-based anti-pneumococcal vaccine, one subject (1/19) was administered with polysaccharide-based anti-pneumococcal vaccine. Four subjects (4/19,) rejected the recommended vaccination. Although all vaccinated subjects responded to the vaccination with a significant specific IgG increase, a secondary drop in specific IgG levels was observed in one subject (1/6,) after an anti-TET vaccine.

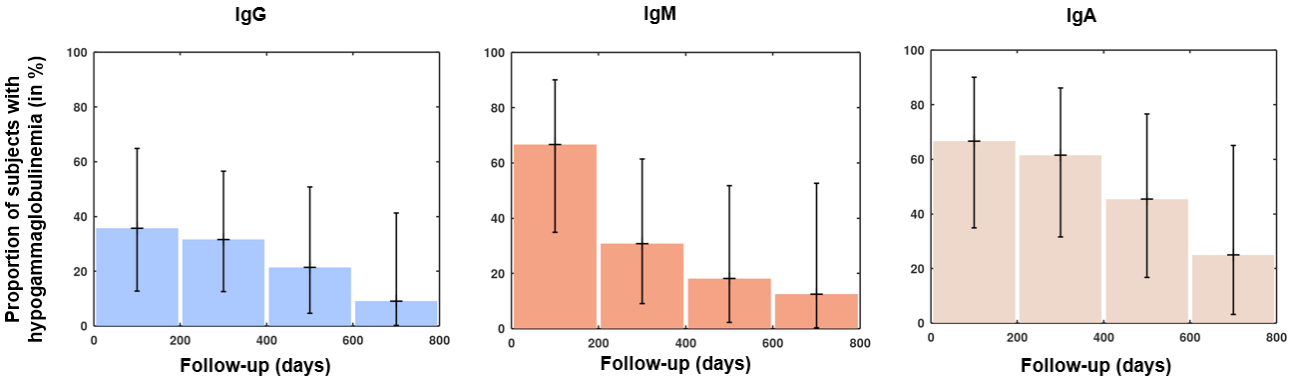

Fig. 1. The percentage of subjects with IgG, IgA IgM hypogammaglobulinemia after B-NHL treatment with single RTX dose included in chemotherapeutic protocols (follow-up period of eight hundred days after the end of treatment). The bar graphs display the proportion of individual subjects with immunoglobulin class level decrease below the lower reference range along with the corresponding 95% confidence intervals for four follow-up intervals. The horizontal line represents the median, the top end of the box represents the upper quartile Q1, while the lower end of the box represents the lower quartile Q3. The whisker above the box plot extends from the upper quartile to the highest actual value (75th percentile + 1.5 (IQR). The whisker below the box plot extends from the lower quartile to lowest actual value within 25th percentile – 1.5(IQR).

DISCUSSION

We aimed to describe B cell reconstitution and immunoglobulin levels values development after pediatric B-NHL protocols, which included a single RTX dose, after the end of B-NHL treatment. Basically, due to a lack of published data, we were unable to compare our results to the results of RTX lacking pediatric B-NHL treatment protocols[33-36]. We do not deny the immunosuppressive and cytotoxic effect of corticosteroids, alkylating agents, and folic acid antagonists[11,28,41,42,54-58]. However, based on the published data, RTX appeared to be the pivotal agent associated with long-term B-cell abnormalities and consequently the antibody deficiency development in patients treated with RTX-containing protocols[9,46,59-64].

The reconstitution kinetics of the cellular and humoral immunity parameters after treatment protocols that include RTX appears to be more altered in children with underlying malignant diseases than in those with autoimmune diseases[14]. Furthermore, patients with malignant diseases treated with RTX appear to be more prone to clinical manifestation of immunodeficiency than RTX-treated patients with autoimmune diseases[14,65]. Also, younger age appears to favor post-RTX B lymphocyte reconstitution[12,66-68].

B cell compartment reconstitution

The median for B cell reconstitution in our cohort was three months after EOT. The majority of reports on B-cell subset reconstitution in pediatric and adolescent patients after treatment regimens that included RTX focused on cohorts with autoimmune diseases. In these, the timing of B cell reconstitution was observed, similar to our study results, as between 6–12 months after RTX administration (1–4 doses, 375 mg/m^2) (ref.[14,62,69]). In adults, multiple RTX dose schemas led to prolonged 12–18-month immunorestitution[14,61,68,70,71]. Semsenchova et al. observed the first B cell reconstitution in pediatric B-NHL patients four month after RTX administration[12,66,67]

In the line with published observations, the B cell subset proportion in our cohort dynamically changed over the FU period reflecting the B cell subset development pattern in infancy[21].

Over the newborn period and infancy, lymphocyte and total B cell absolute counts reach peak values between the first and fifth month of life continuously decreasing until adulthood[21]. Immature-transitional and naïve B cells counts culminate between the first and eleventh month of age, progressively decreasing until adulthood[21,72,73]. Switched memory B cells increase from the second month of age, remaining stable up to five years of age but then decreasing to adulthood values[21,72,73].

Anolik et al. described transitional immature B lymphocyte subset dominance and delayed B cell memory pool recovery after RTX administration, stressing the similarities between ontogenic B cell development, B cell repopulation after allogenic stem cell transplantation, and B cell reconstitution after RTX administration[2]. Identically, in our cohort, transitional and naïve B cells dominated in the first FU interval, slowly decreasing over the FU intervals inversely followed by marginal zone B lymphocyte increase. Our data revealed a continuous increase in switched memory B cells over the first three FU intervals. In contrast to our findings, Semsechenkova at al. found prolonged switched memory B cell suppression in pediatric B-NHL patients treated with protocol that included multiple RTX doses[12].

Hypogammaglobulinemia

According to the literature, up to 30% to 50% (ref.[9,14,68]) of children experience transient or prolonged hypogammaglobulinemia after administration of treatment protocols that included RTX irrespective of normal preexisting IgG levels[9,14,66].

In the first six months (I. FU interval) after the EoT, the immunoglobulin level decrease under the age-adjusted lower reference range in our cohort was 36% of subjects for IgG, 67% in IgA and 67% in IgM (Table 3). Prolonged IgG (9%), IgA (25%), and IgM (13%) hypogammaglobulinemia persisted 800 days after EoT (Table 3). In adults, Casulo et al. reported 39% IgG hypogammaglobulinemia after lymphoma EoT (ref.[25]). Labrosse et al. observed prolonged IgG (26.7%), IgA (22.0%) and IgM (51.2%) hypogammaglobulinemia in a median evaluation of 2.9 years[66]. In contrast to Labrosse et al, instead of IgG, IgA hypogammaglobulinemia dominated among our patients over the FU periods[66].

Decreased IgG production after RTX administration is believed to be affected by SLP depletion, reduction of LLP precursors (class-switched B cells) together with limited LLP survival[60]. Concerning the IgA pool, the IgA production is influenced by LLP in the BM and IgA producing SLP of the intestinal[17,74]. IgM decrease after RTX administration is predominately attributed to IgM-producing SLP depletion[66].

In contrast to published reports, prolonged or failed B-cell lymphocyte subset reconstitution, even long after previous RTX therapy, was not observed in our cohort[9,15,29,32,60,66,75]. No profound IgG decrease under 4 g/L or two standard deviations below the age group means was observed in our patients. However, the literature predominately focuses on the prolonged impact of treatment protocols with multiple RTX doses[60].

Low IgG levels before RTX administration were associated with prolonged IgG hypogammaglobulinemia and increased risk of serious infection after treatment with protocols that included RTX (ref.[9,32,6640,59,75,76]).

Because of the incomplete data on immunoglobulin levels at the time of B-NHL diagnosis we did not evaluate the pretreatment immunoglobulin levels in our trial. Furthermore, we could not exclude the fact that the data at the time of diagnosis could have already been influenced by the disease itself.

Specific antibody response

In our cohort of the ten subjects who received the protein-based anti-tetanus vaccine, four showed a positive post-vaccination response followed by prolonged anti-TET IgG decrease. Retrospectively observed mild IgG, IgA, and IgM hypogammaglobulinemia was present at the time of vaccination in these subjects. These findings support the recommendation of long-term immunology FU in B-NHL patients after the EoT.

There is a paucity of published data on the post-vaccination response after treatment that included RTX in children. Adults with hematological malignancies had a decreased post-vaccination response to the influenza vaccine[77,78]. However, impaired humoral response to the influenza vaccine was identically detected in lymphoma patients without RTX treatment, implying the importance of underlying disease and therapy regime[79,80]. Cha et al. found reduced B c7ell and preserved T cell proliferation response to tetanic toxoid stimuli in adult B-NHL DLBCL patients treated with the B-NHL protocol that included RTX (ref.[81]). In one study, despite long-lasting peripheral B cell depletion, the antigen-specific B cell response was preserved[81]. In addition, the T cell compartment is also implicated, where impaired antigen presentation could then contribute to antibody deficiency manifestation despite the temporarily preserved LLP Ab production after RTX administration[9,26,82].

Infection risk

The subjects with a prolonged IgG decrease in our cohort experienced neither recurrent respiratory tract infection nor severe infection courses with one exception. The infection burden remained low with uncomplicated infection outbreaks. Immunodeficiency was ruled out in the single subject with periproctal abscess.

According to the previous findings, therapeutic protocols that included rituximab seemed to increase the infection risk in children with autoimmune diseases and malignancies[64,66,75,82]. Predominately the IgG decrease was associated with an increased risk of clinically significant infection courses[66,75]. Labrosse et al. described a serious infection course in 23.7% (ref.[66]) patients after treatment schemas with RTX. However, the pediatric group was heterogeneous including autoimmune diseases, malignancies, and Epstein-Barr virus-associated posttransplant lymphoproliferative disease[66,83].

We speculate that the administration of prophylactic ATB in the first vulnerable three to six months after the EoT could be beneficial in decreasing the infection burden. We believe that the absence of profound prolonged IgG hypogammaglobulinemia together with a mostly preserved specific antibody response to antigen led to a lower number of infection courses in our cohort.

Secondary malignancies

In line with Fleury et al, there was no secondary malignancy in our cohort over the FU period[84]. However, only a small cohort over a limited time span was evaluated.

Inborn errors of immunity in hematological malignancies

Cancer, with a prevalence of hematopoietic malignancies, represents the second highest cause of death in children and adults with IEI. Incidentally B-NHL manifestation could precede the IEI diagnosis where antibody deficiency after B NHL treatment could be misdiagnosed as secondary immunodeficiency in these patients[85,86].

Owing to the satisfactory laboratory humoral and cellular immunity parameters for reconstitution and clinical outcome, no further investigation into inborn errors of immunity was conducted in our cohort[51-53]. Labrosse et al. identified in a heterogeneous cohort of 207 pediatric patients treated with a protocol that included RTX, 4.3% of patients with IEI (ref.[66]).

CONCLUSION

A single RTX dose included in therapeutic B-NHL protocols appeared not to be associated with increased severe infection courses in pediatric and adolescent B-NHL

patients after the end of B-NHL treatment. Mild hypogammaglobulinemia remained clinically silent, tending to restore over time. Nonetheless, because of the persisting risk of development of secondary immunodeficiency in B-NHL patients with a history of anti CD20 targeted treatment, expert consensus recommendation on the monitoring of humoral and cellular laboratory features and immunodeficiency signs would be advantageous.

ABBREVIATION

BFM, Berlin-Frankfurt-Münster, BM, Bone-marrow, BCTT, B cell-targeted therapies, CD, Cluster of differentiation, EoT, End of treatment, FU, Follow-up, HiB, *Hemophilus influenzae type b*, IEI, Inborn error of immunity, IGRT, Immunoglobulin replacement therapy, IQR, Interquartile range, LLP, Long-lived plasmablasts, B-NHL, B Non-Hodgkin Lymphoma, NHL BFM, Non-Hodgkin Lymphom Berlin Frankfurt Munster, PCP, Pneumococcal capsular polysaccharide, PID, Primary immunodeficiency, RTX, Rituximab, SLP, Short-lived plasmablasts, TET, Tetanic Toxoid.

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REFERENCES

1. Harker-Murray PD, Pommert L, Barth MJ. Novel Therapies Potentially Available for Pediatric B-Cell Non-Hodgkin Lymphoma. J Natl Compr Canc Netw 2020;18(8):1125-34. doi: 10.6004/jnccn.2020.7608
2. Anolik JH, Friedberg JW, Zheng B, Barnard J, Owen T, Cushing E, Kelly J, Milner E C, Fisher RI, Sanz I. B cell reconstitution after rituximab treatment of lymphoma recapitulates B cell ontogeny. Clin Immunol 2007;122(2):139-45. doi: 10.1016/j.clim.2006.08.009
3. Minard-Colin V, Aupérin A, Pillon M, Burke GAA, Barkauskas DA, Wheatley K, Delgado RF, Alexander S, Uyttebroeck A, Bollard CM. Rituximab for High-Risk, Mature B-Cell Non-Hodgkin's Lymphoma in Children. N Engl J Med 2020;382(23):2207-19. doi: 10.1056/NEJMoa1915315
4. Bollard CM, Lim MS, Gross TG. Committee CN-HL. Children's Oncology Group's 2013 blueprint for research: non-Hodgkin lymphoma. Pediatr Blood Cancer 2013;60(6):979-84. doi: 10.1002/pbc.24416
5. Minard-Colin V, Brugières L, Reiter A, Cairo MS, Gross TG, Woessmann W, Burkhardt B, Sandlund J T., Williams D, Pillon M. Non-Hodgkin Lymphoma in Children and Adolescents: Progress Through Effective Collaboration, Current Knowledge, and Challenges Ahead. J Clin Oncol 2015;33(27):2963-74. doi: 10.1200/JCO.2014.59.5827
6. Burkhardt B, Oschlies I, Klapper W, Zimmermann M, Woessmann W, Meinhardt A, Landmann E, Attarbaschi A, Niggli F, Schrappe M. Non-Hodgkin's lymphoma in adolescents: experiences in 378 adolescent NHL patients treated according to pediatric NHL-BFM protocols. Leukemia 2011;25(1):153-60. doi: 10.1038/leu.2010.245
7. Meinhardt A, Burkhardt B, Zimmermann M, Borkhardt A, Kontny U, Klingebiel T, Berthold F, Janka-Schaub G, Klein C, Kabickova E, Klapper W, Attarbachi A, Schrappe M, Reiter A. Group, B.-F.-M. 2010 Phase II window study on rituximab in newly diagnosed pediatric mature B-cell non-Hodgkin's lymphoma and Burkitt leukemia B J Clin Oncol 2010;28(19):3115-21. doi: 10.1200/JCO.2009.26.6791
8. Bethke M, Varga G, Weinhage T, Sabharwal H, Mellgren K, Randau G, Rolfing M, Wittkowski H, Foell D, Michgehl U, Burghardt B. Patient parameters and response after administration of rituximab in pediatric mature B-cell non-Hodgkin lymphoma. Pediatr Blood Cancer 2022;69(4):e29514. doi: 10.1002/pbc.29514
9. Christou EAA, Giardino G, Worth A, Ladomenou F. Risk factors predisposing to the development of hypogammaglobulinemia and infections post-Rituximab. Int Rev Immunol 2017;36(6):352-59. doi: 10.1080/08830185.2017.1346092
10. Kaplan B, Kopyltsova Y, Khokhar, Lam F, Bonagura V. Rituximab and immune deficiency: case series and review of the literature. J Allergy Clin Immunol Pract 2014;2(5):594-600. doi: 10.1016/j.jaip.2014.06.003
11. Kaplan B, Bonagura VR. Secondary Hypogammaglobulinemia: An Increasingly Recognized Complication of Treatment with Immunomodulators and After Solid Organ Transplantation. Immunol Allergy Clin North Am 2019;39(1):31-47. doi: 10.1016/j.iac.2018.08.005
12. Semchenkova A, Zhogov V, Rudneva A, Potapenko L, Plyasunova S, Miakova N, Samochatova E, Maschan A, Popov A. Immune reconstitution following rituximab-based immunochemotherapy in pediatric patients with B-cell non-Hodgkin lymphomas. Leuk Lymphoma 2022;63(1):217-21. doi: 10.1080/10428194.2021.1998486
13. Pescovitz MD. Rituximab, an anti-cd20 monoclonal antibody: history and mechanism of action. Am J Transplant 2006;6(5 Pt 1):859-66. doi: 10.1111/j.1600-6143.2006.01288.x
14. Worch J, Makarova O, Burkhardt B. Immunreconstitution and infectious complications after rituximab treatment in children and adolescents: what do we know and what can we learn from adults? Cancers (Basel) 2015;7(1):305-28. doi: 10.3390/cancers7010305
15. Wijetilleka S, Mukhtyar C, Jayne D, Ala A, Bright P, Chinoy H, Harper L, Kazmi M, Kiani-Alikhan S, Li C Misbach S, Oni L, Price-Kuehne F, Salama A, Workman S, Wrench D, Karim MY. Immunoglobulin replacement for secondary immunodeficiency after B-cell targeted therapies in autoimmune rheumatic disease: Systematic literature review. Autoimmun Rev 2019;18(5):535-41. doi: 10.1016/j.autrev.2019.03.010
16. Khojah A M, Miller ML, Klein-Gitelman M S, Curran ML, Hans V, Pachman LM, Fuleihan RL. Rituximab-associated Hypogammaglobulinemia in pediatric patients with autoimmune diseases. Pediatr Rheumatol Online J 2019;17(1):61. doi: 10.1186/s12969-019-0365-y
17. Ottaviano G, Marinoni M, Graziani S, Sibson K, Barzaghi F, Bertolini P, Chini L, Corti P, Cancrini C, D'Alba I, Gabelli M, Gallo V, Giancotta C, Giordano P, Lassandro G, Martire B, Angarano R,Mastrodicasa E, Bava C, Miano M, Naviglio S, Verzegnassi F, Saracco P, Trizzino A, Biondi A, Pignata C, Moschese V. Rituximab Unveils Hypogammaglobulinemia and Immunodeficiency in Children with Autoimmune Cytopenia. J Allergy Clin Immunol Pract 2020;8(1):273-82. doi: 10.1016/j.jaip.2019.07.032
18. Harms Pritchard G, Pepper M. Memory B cell heterogeneity: Remembrance of things past. J Leukoc Biol 2018;103(2):269-74. doi: 10.1002/JLB.4MR0517-215R

19. Caraux A, Klein B, Paiva B, Bret C, Schmitz A, Fuhler GM, Bos NA, Johnsen H E, Orfao A, Perez-Andres, M. Network MS. Circulating human B and plasma cells. Age-associated changes in counts and detailed characterization of circulating normal CD138- and CD138+ plasma cells. Haematologica 2010;95(6):1016-20. doi: 10.3324/haematol.2009.018689
20. Perez-Andres M, Paiva B, Nieto WG, Carau A, Schmitz A, Almeida J, Vogt RF, Marti GE, Rawstron AC, Van Zelm MC,Van Dongen JJ, Johnsen HE, Klein B, Orfao A. Primary Health Care Group of Salamanca for the Study of MBL Human peripheral blood B-cell compartments: a crossroad in B-cell traffic. Cytometry B Clin Cytom 2010;78(Suppl 1):S47-60. doi: 10.1002/cyto.b.20547
21. Blanco E, Pérez-Andrés M, Arriba-Méndez S, Contreras-Sanfeliciano T, Criado I, Pelak O, Serra-Caetano, A, Romero A, Puig N, Remesal A, Torres Canziales J, López-Granados E, Kalina T, Sousa AE,van Zelm M, Van der Burg M, van Dongen JJM, Orfao A, Group EP Age-associated distribution of normal B-cell and plasma cell subsets in peripheral blood. J Allergy Clin Immunol 2018;141(6):2208-19.e16. doi: 10.1016/j.jaci.2018.02.017
22. Shapiro-Shelef M, Calame K. Regulation of plasma-cell development. Nat Rev Immunol 2005;5(3):230-42. doi: 10.1038/nri1572
23. Chang HD, Tokoyoda K, Radbruch A. Immunological memories of the bone marrow. Immunol Rev 2018;283(1):86-98. doi: 10.1111/imr.12656
24. Malney DG, Smith B, Rose A. Rituximab: mechanism of action and resistance. Semin Oncol 2002;29(1 Suppl 2):2-9.
25. Casulo C, Maragulia J, Zelenetz AD. Incidence of hypogammaglobulinemia in patients receiving rituximab and the use of intravenous immunoglobulin for recurrent infections. Clin Lymphoma Myeloma Leuk 2013;13(2):106-11. doi: 10.1016/j.clml.2012.11.011
26. Lim SH, Zhang Y, Wang Z, Varadarajan R, Periman P, Esler WV. Rituximab administration following autologous stem cell transplantation for multiple myeloma is associated with severe IgM deficiency. Blood Mar 2004;103(5):1971-2. doi: 10.1182/blood-2003-12-4331
27. Nishio M, Endo T, Fujimoto K, Sato N, Sakai T, Obara M, Kumano K, Minauchi K, Koike T. Persistent panhypogammaglobulinemia with selected loss of memory B cells and impaired isotype expression after rituximab therapy for post-transplant EBV-associated autoimmune hemolytic anemia. Eur J Haematol 2005;75(6):527-9. doi: 10.1111/j.1600-0609.2005.00552.x
28. Otani IM, Lehman HK, Jongco AM, Tsao LR, Azar AE, Tarrant TK, Engel E, Walter J E, Truong TQ, Khan D A, Ballow M, Cunningham-Rundles C, Lu H, Kwan M, Barmettler S. Practical guidance for the diagnosis and management of secondary hypogammaglobulinemia: A Work Group Report of the AAAAI Primary Immunodeficiency and Altered Immune Response Committees. J Allergy Clin Immunol 2022;149(5):1525-60. doi: 10.1016/j.jaci.2022.01.025
29. Patel SY, Carbone J, Jolles S. The Expanding Field of Secondary Antibody Deficiency: Causes, Diagnosis, and Management. Front Immunol 2019;10:33. doi: 10.3389/fimmu.2019.00033
30. Roberts DM, Jones RB, Smith RM, Alberici F, Kumaratne DS, Burns S, Jayne DR. Immunoglobulin G replacement for the treatment of infective complications of rituximab-associated hypogammaglobulinemia in autoimmune disease: a case series. J Autoimmun 2015;57:24-9. doi: 10.1016/j.jaut.2014.11.004
31. Jolles S, Chapel H, Litzman J. When to initiate immunoglobulin replacement therapy (IGRT) in antibody deficiency: a practical approach. Clin Exp Immunol 2017;188(3):333-41. doi: 10.1111/cei.12915
32. Barmettler S, Price C. Continuing IgG replacement therapy for hypogammaglobulinemia after rituximab-for how long? J Allergy Clin Immunol 2015;136(5):1407-9. doi: 10.1016/j.jaci.2015.06.035
33. Martín Ibáñez I, Arce Casas A, Cruz Martínez O, Estella Aguado J, Martín Mateos MA. Humoral immunity in pediatric patients with acute lymphoblastic leukaemia. Allergol Immunopathol (Madr) 2003;31(6):303-10.
34. Lehrnbecher T, Schubert R, Behl M, Koenig M, Rose M. A, Koehl U, Meisel R, Laws HJ. Impaired pneumococcal immunity in children after treatment for acute lymphoblastic leukaemia. Br J Haematol 2009;147(5):700-5. doi: 10.1111/j.1365-2141.2009.07903.x
35. Harrington DS, Weisenburger DD, Purtilo DT. Malignant lymphoma in the X-linked lymphoproliferative syndrome. Cancer 1987;59(8):1419-29. doi: 10.1002/1097-0142(19870415)59:8<1419::aid-cncr2820590807>3.0.co,2-p
36. Del Mistro A, Laverda A, Calabrese F, De Martino M., Calabri G, Cogo P, Cocchi P, D'Andrea E De Rossi A, Giaquinto C. Primary lymphoma of the central nervous system in two children with acquired immune deficiency syndrome. Am J Clin Pathol 1990;94(6):722-8. doi: 10.1093/ajcp/94.6.722
37. Seidemann K, Tiemann M, Henze G, Sauerbrey A, Müller S, Reiter A. Therapy for non-Hodgkin lymphoma in children with primary immunodeficiency: analysis of 19 patients from the BFM trials. Med Pediatr Oncol 1999;33(6):536-44. doi: 10.1002/(sici)1096-911x(199912)33:6<536::aid-mpo3>3.0.co,2-z
38. Bhakta N, Liu Q, Ness KK, Baassiri M, Eissa H, Yeo F, Chemaitilly W, Ehrhardt MJ, Bass J, Bishop MW, Shelton K, Lu L, Huang S, Li Z, Caron E, Lanctot J, Howel C, Folse T, Joshi V, Green DM, Muldrooney DA, Armstrong GT, Krull KR, Brinkman TM,Khan RB,Srivastava DK, Hudson MM, Yasui Y, Robinson LL. The cumulative burden of surviving childhood cancer: an initial report from the St Jude Lifetime Cohort Study (SJLIFE). Lancet 2017;390(10112):2569-82. doi: 10.1016/S0140-6736(17)31610-0
39. Mulrooney DA., Hyun G, Ness KK, Bhakta N, Pui CH, Ehrhardt MJ, Krull KR, Crom DB, Chemaitilly W, Srivastava DK. The changing burden of long-term health outcomes in survivors of childhood acute lymphoblastic leukaemia: a retrospective analysis of the St Jude Lifetime Cohort Study. Lancet Haematol 2019;6(6):e306-e316. doi: 10.1016/S2352-3026(19)30050-X
40. Ehrhardt MJ, Hochberg J, Bjornard KL, Brinkman TM. Long-term survivors of childhood, adolescent and young adult non-Hodgkin lymphoma. Br J Haematol 2019;185(6):1099-110. doi: 10.1111/bjh.15775
41. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol 2008;8(1):59-73. doi: 10.1038/nri2216
42. Chen J, Yang N, Liu H, Yao H, Wang J, Yang Y, Zhang W. Immunological effects of a low-dose cytarabine, aclarubicin and granulocyte-colony stimulating factor priming regimen on a mouse leukemia model. Oncol Lett 2018;16(3):3022-28. doi: 10.3892/ol.2018.9018
43. Di Francia R, Crisci S, De Monaco A., Cafiero C, Re A, Iaccarino G, De Filippi R, Frigeri F, Corazzelli G, Micera A, Pinto A. Response and Toxicity to Cytarabine Therapy in Leukemia and Lymphoma: From Dose Puzzle to Pharmacogenomic Biomarkers. Cancers (Basel) 2021;13(5):966. doi: 10.3390/cancers13050966
44. Tacar O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol 2013;65(2):157-70. doi: 10.1111/j.2042-7158.2012.01567.x
45. Marco H,Smith RM, Jone RB, Guerry MJ, Catapano F Burns S, Chaudhry AN, Smith KG, Jayne DR. The effect of rituximab therapy on immunoglobulin levels in patients with multisystem autoimmune disease. BMC Musculoskelet Disord 2014;15:178. doi: 10.1186/1471-2474-15-178
46. Roberts DM, Jones RB, Smith RM, Alberic F, Kumaratne DS, Burns S, Jayne DR. Rituximab-associated hypogammaglobulinemia: incidence, predictors and outcomes in patients with multi-system autoimmune disease. J Autoimmun 2015;57:60-5. doi: 10.1016/j.jaut.2014.11.009
47. Rosolen A., Perkins SL, Pinkerton CR, Guillerman RP, Sandlund JT, Patte C, Reiter A, Cairo MS. Revised International Pediatric Non-Hodgkin Lymphoma Staging System. J Clin Oncol 2015;33(18):2112-8. doi: 10.1200/JCO.2014.59.7203
48. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J. Lymphoma classification--from controversy to consensus: the R.E.A.L. and WHO Classification of lymphoid neoplasms. Ann Oncol 2000;11(Suppl 1):3-10.
49. Murphy SB. Classification, staging and end results of treatment of childhood non-Hodgkin's lymphomas: dissimilarities from lymphomas in adults. Semin Oncol 1980;7(3):332-9.
50. Morbach H, Eichhorn EM, Liese JG, Girschick HJ. Reference values for B cell subpopulations from infancy to adulthood. Clin Exp Immunol 2010;162(2):271-9. doi: 10.1111/j.1365-2249.2010.04206.x
51. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, Cunningham-Rundles C, Etzioni A, Franco J L., Holland SM, Klein C, Morio T, Ochs HD, Oksenhendler E, Puck J, Torgerson TR, Casanova JL, Sullivan KE, Tangye SG. Human Inborn Errors of Immunity: 2019 Update of the IUIS Phenotypical Classification. J Clin Immunol 2020;40(1):66-81. doi: 10.1007/s10875-020-00758-x

52. Gasteiger LM, Robinson PN, Pazmand J, Boztug K, Seppänen MRJ, Seidel MG; Registry Working Party of the European Society for Immunodeficiencies (ESID). Supplementation of the ESID registry working definitions for the clinical diagnosis of inborn errors of immunity with encoded human phenotype ontology (HPO) terms. J Allergy Clin Immunol Pract 2020;8(5):1778. doi: 10.1016/j.jaip.2020.02.019
53. Seide MG, Kindle G, Gathmann B, Quint I, Buckland M, van Montfrans J, Scheible R, Rusch S, Gasteiger L, M, Grimbacher B, Mahlaoui N, Ehl S, Collaborators ERWPA The European Society for Immunodeficiencies (ESID) Registry Working Definitions for the Clinical Diagnosis of Inborn Errors of Immunity. J Allergy Clin Immunol Pract 2019;7(6):1763-70. doi: 10.1016/j.jaip.2019.02.004
54. Giles A J., Hutchinson MND, Sonnemann HM, Jung J, Fecci PE, Ratnam NM, Zhang W, Song H, Bailey R, Davis D, Reid CM, Park DM,Gilbert MR. Dexamethasone-induced immunosuppression: mechanisms and implications for immunotherapy. J Immunother Cancer 2018;6(1):51. doi: 10.1186/s40425-018-0371-5
55. Nived P, Pettersson Å, Jönsson G, Bengtsson AA, Settergren B, Skattum L, Johansson Å, Kapetanovic MC. Methotrexate reduces circulating Th17 cells and impairs plasmablast and memory B cell expansions following pneumococcal conjugate immunization in RA patients. Sci Rep 2021;11(1):9199. doi: 10.1038/s41598-021-88491-2
56. Genestier L, Paillot R, Fournel S, Ferraro C, Miossec P, Revillard JP. Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells. J Clin Invest 1998;102(2):322-8. doi: 10.1172/JCI2676
57. Rozkova D, Horvath R, Bartunkova J, Spisek R. Glucocorticoids severely impair differentiation and antigen presenting function of dendritic cells despite upregulation of Toll-like receptors. Clin Immunol 2006;120(3):260-71. doi: 10.1016/j.clim.2006.04.567
58. Kuppner MC, Bleifuss E, Noessner E, Mocikat R, Hesler C, Mayerhofer C. Differential effects of ifosfamide on dendritic cell-mediated stimulation of T cell interleukin-2 production, natural killer cell cytotoxicity and interferon-gamma production. Clin Exp Immunol 2008;153(3):429-38. doi: 10.1111/j.1365-2249.2008.03708.x
59. Gottenberg JE, Ravaud P, Bardin T, Cacoub P, Cantagrel A, Combe B, Dougados M, Flipo RM, Godeau B, Guillevin L, Le Löet X, Hachulla E, Schaeverbeke T, Sibilia J, Baron G, Mariette X. AutoImmunity and Rituximab registry and French Society of Rheumatology Risk factors for severe infections in patients with rheumatoid arthritis treated with rituximab in the autoimmunity and rituximab registry. Arthritis Rheum 2010;62(9):2625-32. doi: 10.1002/art.27555
60. Ghielmini M, Schmitz SF, Cogliatti SB, Pichert G, Hummerjohann J, Waltzer U. Post-rituximab immunoglobulin M (IgM) hypogammaglobulinemia. Autoimmun Rev 2020;19(3):102466. doi: 10.1016/j.autrev.2020.102466
61. Ghielmin M, Schmitz SF, Cogliatti SB, Pichert G, Hummerjohann J, Waltzer U, Fey MF, Betticher DC, Martinelli G, Peccatori F, Hess U, Zucca E, Stupp R, Kovacsovics T, Helg C, Lohri A, Bargetzi M,Vorobiof D, Cerny T. Prolonged treatment with rituximab in patients with follicular lymphoma significantly increases event-free survival and response duration compared with the standard weekly x 4 schedule. Blood 2004;103(12):4416-23. doi: 10.1182/blood-2003-10-3411
62. Rao A, Kelly M, Musselman M, Ramadas J, Wilson D, Grossman W, Shenoy S. Safety, efficacy, and immune reconstitution after rituximab therapy in pediatric patients with chronic or refractory hematologic autoimmune cytopenias. Pediatr Blood Cancer 2008;50(4):822-5. doi: 10.1002/pbc.21264
63. Venhoff N, Effelsberg NM, Salzer U, Warnatz K, Peter HH, Lebrecht D, Schlesie M, Voll RE. Impact of rituximab on immunoglobulin concentrations and B cell numbers after cyclophosphamide treatment in patients with ANCA-associated vasculitides. PLoS One 2012;7(5):e3762. doi: 10.1371/journal.pone.0037626
64. Makatsori M, Kiani-Alikhan S, Manson AL, Verma N, Leandro M, Gurugama NP, Longhurst HJ, Grigoriadou S, Buckland M, Kanfer E, Hanson S, Ibrahim MA, Grimbacher B, Chee R, Seneviratne SL. Hypogammaglobulinaemia after rituximab treatment-incidence and outcomes. QJM 2014;107(10):821-8. doi: 10.1093/qjmed/hcu094
65. Pearso ADJ, Scobie N, Norga K, Ligas F, Chiodin D, Burke A, Minard-Colin V, Adamson P, Marshall LV, Balakumaran A, Benettaib B, Bhargava P, Bollard CM, Bolotin E, Bomken S, Buechner J, Burkhardt B, Caron H, Copland C, Demolis P, Egorov A, Farham M, Zugmaier G, Gross T, Horton-Taylor D, Klapper W, Lesa G, Marcus R, Miles RR, Nottage K, Pacaud L, Ricafort R, Schrappe M, Sterba J, Vezan R, weiner S, Kim S.Y, Reaman G, Vassal G. ACCELERATE and European Medicine Agency Paediatric Strategy Forum for medicinal product development for mature B-cell malignancies in children. Eur J Cancer 2019;110:74-85. doi: 10.1016/j.ejca.2019.01.013
66. Labrosse R, Barmettler S, Derfalvi B, Blincoe A, Cros G, Lacombe-Barrios J, Barsalou J, Yang N, Alrumayyan N, Sinclair J, Ong MS, Camrgo CA, Walter J, Haddad E. Rituximab-induced hypogammaglobulinemia and infection risk in pediatric patients. J Allergy Clin Immunol 2021;148(2):523-32.e8. doi: 10.1016/j.jaci.2021.03.041
67. Deyà-Martínez A, Gordón Y, Molina-Anguita C, Vlagea A, Piquer M, Juan M, Esteve-Solé A, Antón J, Madrid Á, García-García A, Plaza AM, Armanguf T, Alsina L. Single-cycle rituximab-induced immunologic changes in children: Enhanced in neuroimmunologic disease? Neurol Neuroimmunol Neuroinflamm 2020;7(4):e724. doi: 10.1212/NXI.0000000000000724
68. Zarkhin V, Li L, Kambham N, Sigdel T, Salvatierra O, Sarwal MM. A randomized, prospective trial of rituximab for acute rejection in pediatric renal transplantation. Am J Transplant 2008;8(12):2607-17. doi: 10.1111/j.1600-6143.2008.02411.x
69. Willems M, Haddad E, Niaudet P. Koné-Paut I, Bensman A, Cochat P, Deschênes G, Fakhouri F, Leblanc T, Llanas B, Liorat T, Pillet P, Ranchin B, Salomon R, Ulinski T, Bader-Meunier B, French Pediatric-Onset SLE Study GroupRituximab therapy for childhood-onset systemic lupus erythematosus. J Pediatr 2006;148(5):623-27. doi: 10.1016/j.jpeds.2006.01.041
70. Ghielmini M, Rufibach K, Salles G, Leoncini-Franscini L, Léger-Falandry C, Cogliatti S, Fey M, Martinelli G, Stahel R, Lohri A, Ketter N, Wernli M, Cerny T, Schmitz SF. Single agent rituximab in patients with follicular or mantle cell lymphoma: clinical and biological factors that are predictive of response and event-free survival as well as the effect of rituximab on the immune system: a study of the Swiss Group for Clinical Cancer Research (SAKK). Ann Oncol 2005;16(10):1675-82. doi: 10.1093/annonc/mdi320
71. Zarkhin V, Lovelace PA, Li L, Hsieh SC, Sarwal MM. Phenotypic evaluation of B-cell subsets after rituximab for treatment of acute renal allograft rejection in pediatric recipients. Transplantation 2011;91(9):1010-8. doi: 10.1097/TP.0b013e318213df29
72. Piątosa B, Wolska-Kuśnierz B, Pac M, Siewiera K, Gałkowska E, Bernatowska E. B cell subsets in healthy children: reference values for evaluation of B cell maturation process in peripheral blood. Cytometry B Clin Cytom 2010;78(6):372-81. doi: 10.1002/cyto.b.20536
73. van Gent R. van Tilburg CM, Nibbelke EE, Otto SA, Gaiser J F, Janssens-Korpela PL, Sanders EA, Borghans J. A, Wulffraat N M, Bierings MB, Bloem AC, Tesselaar K. Refined characterization and reference values of the pediatric T- and B-cell compartments. Clin Immunol 2009;133(1):95-107. doi: 10.1016/j.clim.2009.05.020
74. Mei HE, Frölich D, Giesecke C, Loddenkemper C, Reiter K, Schmidt S, Feist E, Daridon C, Tony HP, Radbruch A. Steady-state generation of mucosal IgA+ plasmablasts is not abrogated by B-cell depletion therapy with rituximab. Blood 2010;116(24):5181-90. doi: 10.1182/blood-2010-01-266536
75. Barmettler S, Ong MS, Farmer JR, Choi H, Walter J. Association of Immunoglobulin Levels, Infectious Risk, and Mortality With Rituximab and Hypogammaglobulinemia. JAMA Netw Open 2018;1(7):e184169. doi: 10.1001/jamanetworkopen.2018.4169
76. Diwakar L, Gorrie S, Richter A, Chapman O, Dhillon P, Al-Ghanmi F, Noorani S, Krishna MT, Huissoon A Does rituximab aggravate pre-existing hypogammaglobulinaemia? J Clin Pathol 2010;63(3):275-7. doi: 10.1136/jcp.2009.068940
77. Yri OE, Torfoss D, Hungnes O, Tierens A, Waalen K, Nordøy T, Dudman S, Kilander A, Wader KF, Ostenstad B, Ekanger R, Mezer P. Kolstad ARituximab blocks protective serologic response to influenza A (H1N1) 2009 vaccination in lymphoma patients during or within 6 months after treatment. Blood 2011;118(26):6769-71. doi: 10.1182/blood-2011-08-372649
78. Bedognetti D, Zoppoli G, Massucco C, Zanardi E, Zupo S, Bruzzone A, Sertoli MR, Balleari E, Racchi O, Messina M,Caltabiano G, Icardi G, Durando P, Marincola FM, Boccardo F, Ferrarini M, Ansaldi E, DeMaria A. Impaired response to influenza vaccine associated with persistent memory B cell depletion in non-Hodgkin's lymphoma patients treated with rituximab-containing regimens. J Immunol 2011;186(10):6044-55. doi: 10.4049/jimmunol.1004095

79. Rapezzi D, Sticchi L, Racchi O, Mangerini R, Ferraris AM, Gaetani GF. Influenza vaccine in chronic lymphoproliferative disorders and multiple myeloma. Eur J Haematol 2003;70(4):225-30. doi: 10.1034/j.1600-0609.2003.00028.x
80. Feery BJ, Sullivan JR, Hurley TH, Evered MG. Immunization with influenza vaccine in patients with haematological malignant disease. Med J Aust 1977;1(9):292-4. doi: 10.5694/j.1326-5377.1977.tb130704.x
81. Cha Z, Li C, Zang Y, Gu H, Guo H, Li J, Fang Y, Petersen T, Karas RO, Hamilton ML, Qian B. Adaptive B cell responses in rituximab-treated diffuse large B cell lymphoma patients during complete remission. Tumour Biol 2016;37(1):829-35. doi: 10.1007/s13277-015-3872-6
82. Kelesidis T, Daikos G, Boumpas D, Tsiodras S. Does rituximab increase the incidence of infectious complications? A narrative review. Int J Infect Dis 2011;15(1):e2-16. doi: 10.1016/j.ijid.2010.03.025
83. Castagnola E, Dallorso S, Faraci M, Morreale G, Di Martino D, Cristina E, Scarso L, Lanino E. Long-lasting hypogammaglobulinemia following rituximab administration for Epstein-Barr virus-related post-transplant lymphoproliferative disease preemptive therapy. J Hematother Stem Cell Res 2003;12(1):9-10. doi: 10.1089/152581603321210082
84. Fleury I, Chevret S, Pfreundschuh M, Salles G, Coiffier B, van Oer MH, Gisselbrecht C, Zucca E, Herold M, Ghielmini M, Thieblemont C. Rituximab and risk of second primary malignancies in patients with non-Hodgkin lymphoma: a systematic review and meta-analysis. Ann Oncol 2016;27(3):390-7. doi: 10.1093/annonc/mdv616
85. Riaz IB, Faridi W, Patnaik MM, Abraham RS. A Systematic Review on Predisposition to Lymphoid (B and T cell) Neoplasias in Patients With Primary Immunodeficiencies and Immune Dysregulatory Disorders (Inborn Errors of Immunity). Front Immunol 2019;10:777. doi: 10.3389/fimmu.2019.00777
86. Hauck F, Voss R, Urban C, Seidel MG. Intrinsic and extrinsic causes of malignancies in patients with primary immunodeficiency disorders. J Allergy Clin Immunol 2018;141(1):59-68.e4. doi: 10.1016/j.jaci.2017.06.009