Original Article

Rituximab Unveils Hypogammaglobulinemia and Immunodeficiency in Children with Autoimmune Cytopenia

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What is already known about this topic? Postrituximab hypogammaglobulinemia can occur, but the prevalence in children with autoimmune cytopenia is unknown.

What does this article add to our knowledge? Persistent hypogammaglobulinemia after rituximab is associated with delayed B-cell recovery. Risk factors for persistent hypogammaglobulinemia included younger age, better overall response to treatment, and a diagnosis of autoimmune hemolytic anemia or Evans syndrome; an underlying primary immune deficiency was identified more frequently in these children.

How does this study impact current management guidelines? Persistent hypogammaglobulinemia after rituximab in children with autoimmune cytopenia should not always be interpreted as iatrogenic immunologic impairment because it may unveil a diagnosis of primary immune deficiency and should prompt further diagnostic tests.

BACKGROUND: Rituximab (RTX; anti-CD20 mAb) is a treatment option in children with refractory immune thrombocytopenia, autoimmune hemolytic anemia (AHA), and Evans syndrome (ES). Prevalence and clinical course of RTX-induced hypogammaglobulinemia in these patients are poorly known.

OBJECTIVE: To evaluate the prevalence and risk factors for persistent hypogammaglobulinemia (PH) after RTX use.
METHODS: Clinical and immunologic data from children treated with RTX for immune thrombocytopenia, AHA, and ES were collected from 16 Italian centers and 1 UK center at pre-RTX time point (0), +6 months, and yearly, up to 4 years post-

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Abbreviations used
AHA- Autoimmune hemolytic anemia
CVID- Common variable immunodeficiency
ES- Evans syndrome
ITP- Immune thrombocytopenia
PAD- Predominant antibody deficiency
PH- Persistent hypogammaglobulinemia
PID- Primary immunodeficiency
RTX- Rituximab
smB- Switched-memory B

RTX. Patients with previously diagnosed malignancy or primary immune deficiency (PID) were excluded.
RESULTS: We analyzed 53 children treated with RTX for immune thrombocytopenia (n = 36), AHA (n = 13), and ES (n = 4). Median follow-up was 30 months (range, 12-48). Thirty-two percent of patients (17 of 53) experienced PH, defined as IgG levels less than 2 SD for age at last follow-up (>12 months after RTX). Significantly delayed B-cell recovery was observed in children experiencing PH (hazard ratio, 0.55; $P < .05$), and 6 of 17 (35%) patients had unresolved B-cell lymphopenia at last follow-up. PH was associated with IgA and IgM deficiency, younger age at RTX use (51 vs 116 months; $P < .01$), a diagnosis of AHA/ES, and better response to RTX. Nine patients with PH (9 of 17 [53%]) were eventually diagnosed with a PID.
CONCLUSIONS: Post-RTX PH is a frequent condition in children with autoimmune cytopenia; a sizable proportion of patients with post-RTX PH were eventually diagnosed with a PID. In-depth investigation for PID is therefore recommended in these patients. **© 2019 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;■:■-■)**

Key words: *Rituximab; Hypogammaglobulinemia; Cytopenia; Autoimmunity; Primary immunodeficiency*

INTRODUCTION

Immune thrombocytopenia (ITP), autoimmune hemolytic anemia (AHA), and Evans syndrome (ES) are autoimmune conditions that can be successfully treated with immunoglobulins and/or steroids, or may not require any treatment in specific situations (ie, mild symptomatic ITP).[1-6] However, when the cytopenia is symptomatic and refractory to first-line treatments, rituximab (RTX), an mAb anti-CD20, can be used to deplete B cells considered responsible for autoantibody production.[7-10] The rationale for RTX rests on its specificity for B cells expressing CD20. Because both plasma cells and pro-B cells lack CD20 expression, they are not depleted by RTX and can fulfill IgG production and B-cell immune reconstitution, respectively.[11] For this reason, hypogammaglobulinemia is not usually expected after treatment with RTX. However, both a drop in IgM, although temporary,[12,13] and persistent low IgG level in high-risk patients (eg, affected by malignant disorders) have been reported.[14,15] The role of RTX in inducing prolonged low IgG levels is not easily discernible in these patients, because of the heterogeneity of treatments as well as their underlying hemato-oncological disease. Moreover, the clinical significance of hypogammaglobulinemia after RTX is unclear, because a very low incidence of infections has been reported,[16-18] although some authors recommend immunoglobulin replacement therapy to prevent infectious complications.[19,20] A higher number of RTX administrations and lower basal IgG levels have been associated with a higher rate of symptomatic hypogammaglobulinemia in adults.[14] In children, data on reconstitution of B cells and IgG levels are scarce and limited to case reports/series. However, persistent hypogammaglobulinemia (PH) requiring immunoglobulin replacement has been described in children with autoimmune conditions treated with RTX.[21-29] Interestingly, some of these patients presented immunologic and clinical features recalling common variable immunodeficiency (CVID), supporting the hypothesis that RTX-induced B-cell perturbation can unveil a primary immunologic disorder.[30]

The aim of this study was to assess the prevalence and risk factors for PH in a cohort of children who received RTX for autoimmune cytopenia, to assist clinicians in the surveillance and management of these patients.

METHODS

From May 2016 to January 2019 we retrospectively and prospectively collected data on children who were treated with RTX for autoimmune cytopenia (ITP, AHA, and ES) at 16 centers from the Italian Network for Primary Immunodeficiency and at Great Ormond Street Hospital (London, UK). Standard dose of single RTX administration was 375 mg/m^2. Patients younger than 18 years at the time of treatment with RTX with at least 12 months of clinical and immunologic follow-up were included. Children with malignant disorders or primary immunodeficiency (PID) or PID-like phenotype (eg, autoimmune lymphoproliferative syndrome or autoimmune lymphoproliferative syndrome—like conditions) at the time of RTX use were excluded. Pre-RTX and follow-up data at +6, +12, +24, +36, and up to +48 months after the last dose of RTX were retrieved through case report forms. Demographic data, clinical history, IgG, IgM, and IgA levels, and peripheral blood lymphocyte count flow cytometry (CD3$^+$, CD4$^+$, CD8$^+$, CD19$^+$, CD16/56$^+$) were included. Genetic analysis and B-/T-cell extended phenotypic analyses were performed according to clinician decision and/or technical availability. Diagnosis of PID was made when pathogenic mutations were found in genes associated with PIDs or according to the clinical phenotype, classifying patients as reported by the 2017 IUIS Phenotypic Classification.[31]

The study protocol conforms to the ethical guidelines of the 1979 Declaration of Helsinki as reflected in *a priori* approval by the institution's human research committee. Institutional Ethical Committee of the University of Rome Tor Vergata approved the study.

Definitions

Transient hypogammaglobulinemia was defined as low IgG levels (<2 SD for age) with subsequent normalization of IgG values and/or discontinuation of immunoglobulin replacement for those patients who started immunoglobulin replacement therapy. PH was defined as IgG less than 2 SD for age after RTX and no subsequent spontaneous recovery at last available follow-up more than 12 months after RTX. IgA and IgM deficiencies were considered to be present when these immunoglobulin levels were less than 2 SD for age.[32] T- and B-cell immune reconstitution was defined by absolute lymphocyte subset counts within normal range for age at last follow-up.[33]

TABLE I. Clinical and demographic features of 53 children treated with RTX for autoimmune cytopenia

Feature	Value (N = 53), n (%)
Diagnosis	
ITP	36 of 53 (69%)
AHA	13 of 53 (24%)
ES	4 of 53 (7%)
Age at diagnosis (mo)	
Median	72
Range	1-204
Age at RTX (mo)	
Median	114
Range	2-208
Sex	
Male	31 of 53 (58%)
Female	22 of 53 (42%)
RTX doses	
Median	4 doses
Range	1-16
<4 doses	8 of 53 (15%)
4 doses	38 of 53 (73%)
>4 doses	7 of 53 (12%)
Pre-RTX therapies	
HD-IVIG only	3 of 53 (5%)
CS only	2 of 53 (4%)
CS + MMF	1 of 53 (2%)
CS + sirolimus	1 of 53 (2%)
HD-IVIG + CS only	33 of 53 (60%)
HD-IVIG + CS + other*	13 of 53 (27%)
TPO agonist	5 of 53 (9%)
MMF	5 of 53 (9%)
Azathioprine	2 of 53 (4%)
Immunoglobulin anti-IgD	2 of 53 (4%)
Cyclophosphamide	2 of 53 (4%)
Methotrexate	1 of 53 (2%)
Cyclosporin	1 of 53 (2%)
Sirolimus	1 of 53 (2%)
Splenectomy	1 of 53 (2%)
Autoimmunity	
Autoimmune thyroiditis	6 of 53 (11%)
Autoimmune neutropenia	2 of 53 (4%)
Celiac disease	1 of 53 (2%)
Autoimmune hepatitis	1 of 53 (2%)
Crohn disease	1 of 53 (2%)
Anti-ADAMTS13 syndrome	1 of 53 (2%)
Infections	
Hospitalization	7 of 53 (12%)
Recurrent respiratory infections	8 of 53 (15%)

CS, Corticosteroid; *HD-IVIG*, high-dose intravenous immunoglobulin; *MMF*, mycophenolate mofetil; *TPO*, thrombopoietin.
*Patients could receive more than 1 additional therapy after HD-IVIG and CS; therefore, there is no correspondence with the total of patients.

Statistical analysis

Patients' features and demographic characteristics were included in descriptive statistics. Log-rank test was performed to compare B-cell immune reconstitution kinetics. Continuous variables were expressed as median and range and were compared between groups using Student *t* test. The threshold for statistical significance was set to a *P* value less than .05. Univariate analysis using the Fisher exact test was used to investigate potential risk factors for PH. Variables that showed a significant ($P < .05$) association with outcome in univariate analysis were included in logistic regression model for multivariate analysis. Data analysis and statistics were performed using Prism GraphPad software, version 6 (GraphPad Software, Inc, La Jolla, Calif) and Epi Info 7 software (Centers for Disease Control and Prevention, Atlanta, Ga).

RESULTS

Patients' characteristics

We enrolled 53 children who received RTX in the study period. Data on pre-RTX immunoglobulin levels and B cells were available for 40 of 53 (76%) and 39 of 53 (73%) patients, respectively. No determination was available for 6 patients, and 7 patients had an IgG level of greater than 2000 mg/dL, but levels were considered unreliable because they were investigated close to infusion of high-dose intravenous immunoglobulins. Demographic characteristics and clinical features of the study population are presented in Table I. All patients received at least 1 front-line therapy before RTX. Most children (33 of 53 [60%]) were refractory to both steroids and high-dose intravenous immunoglobulins, and RTX was used as a third-line treatment. Conversely, additional immunosuppressive drugs were administered after high-dose intravenous immunoglobulins and/or steroids in 10 patients: a single agent was used in 5 patients, whereas 4 children and 1 child received 2 and 3 immunosuppressive drugs, respectively. Finally, a thrombopoietin analogue (romiplostim) was used before RTX in 5 patients. Most of the patients received a single course of RTX (4 weekly doses at 375 mg/m^2/dose), according to international guidelines.[1,6] However, a small proportion (12%) of patients required more than 1 treatment cycle, up to 4 courses. Eight patients had a history of recurrent respiratory tract infections, and 7 patients required admission for infections (range, 1-3 hospitalizations) before RTX. Considering autoimmune manifestations (other than ITP, AHA, and ES), 23% (12 of 53) of the patients had a history of concomitant autoimmune disease at the time of diagnosis or during follow-up: 6 patients presented with autoimmune thyroiditis, 2 with autoimmune neutropenia, 1 with autoimmune hepatitis, 1 with celiac disease, 1 with Crohn disease, and 1 with anti-ADAMTS13 antibody syndrome. Genetics for PID was performed for 4 patients before RTX use. The *WAS* gene was sequenced in 1 patient, *XIAP* and *SH2D1A* genes in other 2 patients, and autoimmune lymphoproliferative syndrome—causing genes (*FAS*, *FAS ligand*, *Caspase 8*, and *Caspase 10*) were tested in 1 patient. All patients were wild-type for the analyzed genes. No patient presented a significant family history for PIDs.

Hypogammaglobulinemia and immune reconstitution after RTX

After the last RTX course, patients were followed up for 30 months (mean, range 12-48 months). Twenty-nine patients (55%) did not develop hypogammaglobulinemia after RTX. A transitory drop in IgG levels was observed in 7 patients (13%), who started immunoglobulin but subsequently discontinued the treatment and recovered IgG levels within the first year after RTX. However, 16 of 53 (30%) children persistently needed immunoglobulin to maintain adequate IgG levels more than 12

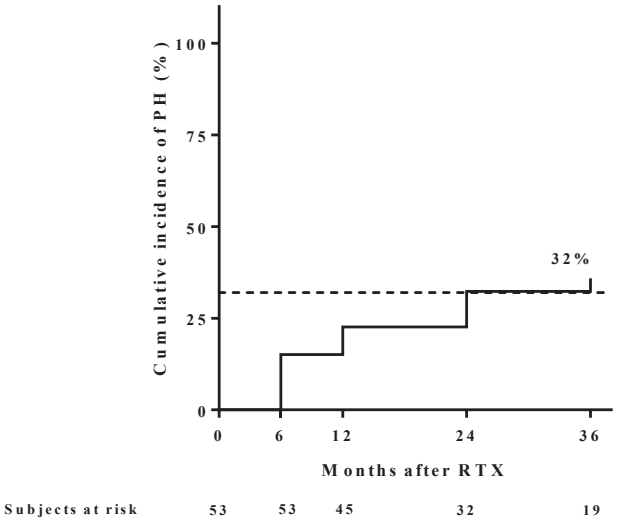

FIGURE 1. Cumulative incidence of PH during follow-up in 53 children treated with RTX.

months after RTX, and 1 patient showed a low IgG level (<2 SD) for age at last follow-up although he did not receive substitutive immunoglobulins because of parental decision, more than 1 year after the treatment. These patients (17 of 53 [32%]) were considered affected by PH, whereas children with normal IgG levels at last follow-up more than 12 months after RTX were defined as patients without PH (Figure 1). Nearly half the patients with PH (8 of 17 [47%]) started immunoglobulin within 6 months after RTX, whereas immunoglobulin treatment was initiated at 12 months or later in 8 patients.

The incidence of IgA and IgM deficiency was also investigated. A similar proportion of patients had IgM levels less than 2 SD for age in the 2 groups before RTX (11% [4 of 36] vs 17% [3 of 17]) (Tables II and III). However, looking at patients with normal IgM levels at enrollment, new-onset hypo-IgM during follow-up was more frequent in children with PH (10 of 11 [91%]) than in the group without PH (8 of 29 [28%]). Timing to observe IgM normalization was also delayed in children with PH (log-rank hazard ratio, 0.12; 95% CI, 0.07-0.35; $P < .0001$; Figure 2, *A*), but it occurred within 12 months after RTX in children without PH. Prevalence of low IgA levels for age was comparable at baseline in both groups (8% [3 of 36] vs 23% [4 of 17]), but after RTX patients with normal IgA levels more frequently developed IgA deficiency in the PH group (80% [8 of 10]) than in the non-PH group (7% [2 of 30]). Significantly, slower IgA recovery occurred after RTX in children with PH (hazard ratio, 0.18; 95% CI, 0.05-0.22; $P < .0001$; Figure 2, *B*) compared with children without PH, who normalized IgA levels within 12 months from treatment. Finally, new-onset combined isotype deficiency (IgM and IgA levels below 2 SD for age) was significantly more frequent (7 of 17 [41%] vs 1 of 36 [3%]; $P < .001$) in patients with PH.

B-cell immune reconstitution was evaluated during follow-up at 5 time points. In the PH group, 6 of 17 patients (35%) did not show complete B-cell recovery; in contrast 34 of 36 (94%) patients with normal IgG levels at last follow-up had normal B cells after RTX. Notably, B-cell immune reconstitution was significantly delayed in the PH group (hazard ratio, 0.55; 95% CI, 0.2-0.65; $P < .05$), whereas 83% of the patients without PH already normalized B-cell count at 12 months after RTX (Figure 3). B-cell memory subsets were investigated in 6 patients with PH, and 5 had low (<2%) $CD27^{+}IgM^{-}IgD^{-}$ switched-memory B (smB) cells.

TABLE II. Comparison of pre-RTX vs post-RTX immunoglobulin levels in 36 children with no or transient hypogammaglobulinemia

	IgG (mg/dL)		IgM (mg/dL)		IgA (mg/dL)	
No.	Pre-RTX	Post-RTX	Pre-RTX	Post-RTX	Pre-RTX	Post-RTX
1	804	903	115	**21**	73	53
2	—	659	—	—	—	—
3	796	816	**41**	**29**	57	75
4	1800	1540	251	157	271	277
5	1280	1230	**44**	**27**	253	233
6	—	630	—	114	—	56
7	1158	880	100	77	259	230
8	—	563	—	**21**	—	71
9	960	850	78	66	156	100
10	**239**	494	86	47	36	33
11	949	919	101	161	105	94
12	748	644	116	61	140	143
13	765	816	108	37	67	134
14	1850	1760	248	**34**	122	**46**
15	1250	1180	114	71	107	119
16	1630	1310	108	57	112	110
17	1510	1370	178	98	78	70
18	960	896	99	53	24	22
19	1096	984	252	69	**16**	106
20	1482	607	128	**37**	137	96
21	1663	1442	98	**17**	**<5**	**<5**
22	1040	1030	146	187	118	141
23	765	655	97	—	83	—
24	1795	1440	66	41	97	118
25	696	518	86	42	107	66
26	1034	1028	116	50	140	173
27	626	695	41	—	155	—
28	—	1260	**31**	**44**	**50**	**47**
29	1230	1280	165	**33**	67	45
30	—	975	88	**27**	337	209
31	—	808	134	76	100	110
32	855	752	66	**34**	200	212
33	519	700	**35**	**31**	38	98
34	1070	1030	133	50	94	99
35	—	916	158	**24**	49	**7**
36	**376**	790	79	92	80	103

Values in bold: Immunoglobulin <2 SD of age-matched controls; cells with em dash: missing data.

Risk factors for persistent hypogammaglobulinemia

We compared the baseline clinical and immunologic characteristics of children with PH vs children without PH (Table IV). Median pre-RTX IgA and IgM levels were lower in the PH group (48 mg/dL vs 103 mg/dL and 62 mg/dL vs 105 mg/dL, respectively). A younger age at diagnosis of autoimmune cytopenia was observed in patients with PH (51 vs 116 months), whereas no significant differences were found when age at treatment, B- and T-lymphocyte counts, and IgG levels at enrollment were compared. Univariate analysis showed that a

TABLE III. Comparison of pre-RTX immunoglobulin levels with post-RTX immunoglobulin levels in 17 children with PH

	IgG (mg/dL)		IgM (mg/dL)		IgA (mg/dL)	
No.	Pre-RTX	Post-RTX	Pre-RTX	Post-RTX	Pre-RTX	Post-RTX
1	1360	**96**	67	**5**	321	**8**
2	331	**462***	**6**	**25**	**23**	**<8**
3	795	**254**	73	**5**	106	**<6**
4	521	**760***	44	**7**	92	**23**
5	—	**285**	—	**5**	—	**13**
6	1720	**442**	81	**1**	52	**17**
7	1260	**291**	87	**7**	**44**	**14**
8	432	**580***	43	**<5**	36	**<6**
9	1280	**867***	**41**	**28**	59	76
10	327	**118**	43	50	**28**	58
11	1520	**278**	56	**19**	**15**	**<5**
12	418	**272**	**16**	**39**	58	**19**
13	1650	**451**	80	**11**	88	**31**
14	—	**608***	—	**3**	—	69
15	—	**210**	—	**<17**	—	**<5**
16	843	**258**	91	**36**	114	110
17	523	**321**	41	**8**	52	**28**

IVIG, Intravenous immunoglobulin.
Values in bold: Immunoglobulin <2 SD of age-matched controls; box with em dash: missing data.
*Immunoglobulin values after IVIG replacement therapy.

diagnosis of AHA/ES vs ITP diagnosis was significantly associated with PH (Table V). Moreover, patients in whom the cytopenia resolved after B-cell depletion were more frequently affected by PH (59% vs 28%; $P < .05$). Also, the occurrence of autoimmune manifestations (other than the original autoimmune cytopenia) correlated with PH. However, total RTX administrations and use of additional immunosuppressants were not associated with a higher risk of PH. On multivariate analysis, AHA/ES and response to RTX were independently associated with occurrence of sustained IgG deficiency (Table V).

Clinical phenotype and diagnosis of PID

No significant infections were documented after treatment with RTX for patients who maintained normal IgG levels during follow-up. In contrast, in the PH group post-RTX infections requiring hospitalization were reported for 5 of 17 patients (29%). Four patients presented with multiple upper and lower airway infections (>4 episodes/y), requiring antibiotic therapy. Peripheral blood EBV reactivation was documented in 2 patients. Also, 1 patient presented with *Pneumocystis jirovecii* pneumonia. Finally, 1 patient suffered from perianal bacterial lesions. Noninfectious complications included EBV-driven lymphoma, additional autoimmune manifestations, and splenomegaly. Among the 17 patients with PH, 9 (53%) were eventually diagnosed as suffering from PID. In 2 children with PH, next-generation sequencing panel analysis allowed the identification of a pathogenic homozygous mutation in the *DCLRE1C* gene and a heterozygous gain-of-function mutation in the *PIK3CD* gene. Other 5 patients presented with the clinical picture of patients with CVID (hypogammaglobulinemia, recurrent infections, IgA and IgM deficiency, low smB cells, and impaired vaccine responses) and 2 patients had a predominant antibody deficiency (PAD), not better specified (no information

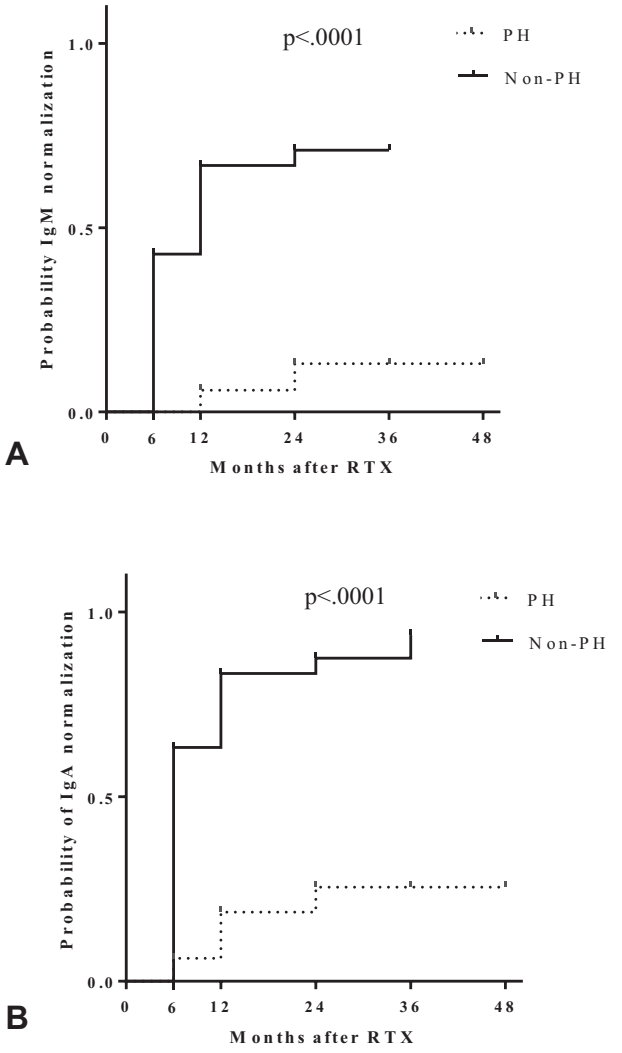

FIGURE 2. (**A**) Log-rank analysis for comparison of IgM normalization post-RTX in children with PH vs children with normal IgG levels (non-PH). (**B**) Log-rank analysis for comparison of IgA normalization post-RTX in children with PH vs children with normal IgG levels (non-PH).

was available on vaccine responses and B-cell subsets). Detailed phenotype and immunologic abnormalities of these 9 patients are presented in Table VI. Single genes (*TACI*, *PRF1*, and *WAS*) and next-generation sequencing panels for multiple genes associated with hypogammaglobulinemia were analyzed in 6 patients, but no abnormalities were observed. Pre-RTX immunologic status was not available in 1 patient, and no data on B cells were available at enrollment for another patient, who, however, had a normal immunoglobulin level before starting anti-CD20 mAb. Six patients had normal IgG and B cells before RTX. Only 2 patients already presented with low immunoglobulins and/or low lymphocyte counts, although they were extensively treated with high-dose steroids (patient 1 and patient 6 in Table VI).

DISCUSSION

RTX is considered an effective treatment for several autoimmune diseases with an acceptable safety profile. However, some

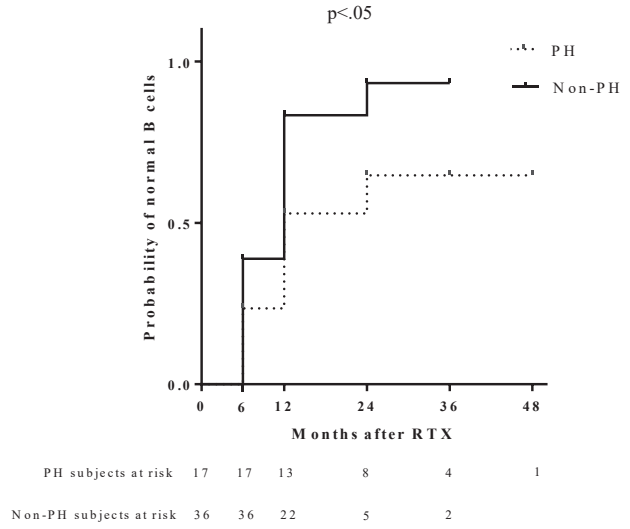

FIGURE 3. Log-rank analysis for comparison of B-cell recovery in children with PH vs children with normal IgG levels after RTX (non-PH).

Immunologic workup is not routinely performed before the administration of RTX. Indeed, more than 80% of patients treated with anti-CD20 mAb for autoimmune diseases are not screened for immunoglobulin deficiency and the risk of hypogammaglobulinemia is not consistently assessed during follow-up. However, when IgG levels are monitored, the prevalence of new-onset hypogammaglobulinemia can be significant (~20%).[34] The lack of standardized follow-up in clinical studies and the use of prophylactic immunoglobulin replacement therapy in the first months after treatment limit the assessment of hypogammaglobulinemia. Studies in adults with autoimmune cytopenia treated with RTX reported an incidence of 6% of severe hypogammaglobulinemia,[9] but data on children are lacking. Rao et al[12] only reported that the nadir of IgG after anti-CD20 administration in children with ITP or AHA is reached at 12 months, but they did not report the proportion of patients with IgG levels less than 2 SD for age and data on longer follow-up. The extended follow-up of our study could have facilitated the diagnosis of late-occurring hypogammaglobulinemia: indeed, for half the patients with PH, low IgG levels were first observed more than 12 months after treatment. This suggests that long-term follow-up is needed to assess the true incidence of

TABLE IV. Patients' features and pre-RTX immunologic phenotype comparison of children with PH vs children without PH

Feature	PH = 17	Non-PH = 36	*P* value
Age at diagnosis, mo (range)	51 (1-167)	116 (4-204)	**<.01**
Age at RTX, mo (range)	95 (2-180)	116 (6-208)	.15
IgG (mg/dL), median (range)	659 (327-1360)	997 (239-1850)	.1
IgA (mg/dL), median (range)	48 (6-321)	103 (4-337)	**<.01**
IgM (mg/dL), median (range)	62 (16-252)	105 (31-251)	**<.01**
$CD3^+/mm^3$, median (range)	1330 (195-2587)	1437 (600-3912)	.3
$CD19^+/mm^3$, median (range)	282 (90-588)	465 (129-2000)	.2

Bold characters represent significant or close to significance cut-off values.

TABLE V. Risk factors associated with the development of PH in children treated with RTX by Fischer exact test analysis and logistic regression model for variables with significant association with PH development in univariate analysis

	Univariate analysis				Logistic regression	
Risk factor	PH = 17	Non-PH = 36	OR (95% CI)	*P* value	OR (95% CI)	*P* value
AHA/ES	59% (10 of 17)	17% (7 of 36)	5.9 (1.7-21)	**<.01**	5.5 (1.1-27)	**<.05**
Age at diagnosis < median	65% (11 of 17)	39% (14 of 36)	2.9 (0.9-10)	.14		
Age at RTX < median	59% (10 of 17)	39% (14 of 36)	2.2 (0.7-6)	.2		
RTX doses > 4	24% (4 of 17)	8% (3 of 36)	2 (0.4-10)	.4		
Response to RTX	59% (10 of 17)	28% (10 of 36)	3.7 (1.1-12)	**<.05**	4.9 (1.6-24)	**<.05**
Autoimmunity	41% (7 of 17)	14% (5 of 36)	4.34 (1.1-16)	**<.05**	4.8 (0.8-28)	**.07**
Additional IS*	29% (5 of 17)	14% (5 of 36)	1.2 (0.4-6)	.7		

HD-IVIG, High-dose intravenous immunoglobulin; *IS*, immunosuppressants; *OR*, odds ratio.
Bold characters represent significant or close to significance cut-off values.
*In addition to steroids and HD-IVIG.

patients can develop hypogammaglobulinemia. We reported the extensively detailed long-term follow-up of 53 children treated for autoimmune cytopenia with mAb anti-CD20, who experienced a 32% prevalence of sustained hypogammaglobulinemia. We showed that prolonged B-cell depletion was associated with IgA and IgM deficiency for a significant proportion of patients and IgG deficiency, overall leading to clinical or genetic diagnosis of PID in about 20% of treated children.

post-RTX hypogammaglobulinemia.

We showed that PH is usually followed by a drop in other immunoglobulin isotype levels and when IgA and IgM levels remain low after 12 months, post-RTX spontaneous improvement is unlikely, suggesting a potential impaired maturation of immunoglobulin-producing cells. This was also suggested by the low smB-cell percentages documented in some patients with PH (5 of 17 [29%]), resembling the immunologic phenotype of

TABLE VI. Clinical and immunologic features of patients with PH and final diagnosis of PID

Patient no.	Pre-RTX phenotype	Post-RTX clinical phenotype	Post-RTX immune phenotype	Final diagnosis
1	AHA, 2 y	Follow-up 36 mo		ARTEMIS
	IgG: 331 mg/dL	Proteus sepsis, EBV-driven Burkitt lymphoma	IgG: 666* mg/dL	
	IgA: 6 mg/dL	CMV reactivation	IgA: <8 mg/dL	
	IgM: 23 mg/dL	ITP	IgM: 25 mg/dL	
	$CD19^+$: 315/mm^3		$CD19^+$: 110/mm^3	
	$CD3^+$: 200/mm^3		$CD3^+$: 3256/mm^3	
	$CD3^+/CD4^+$: 101/mm^3		$CD3^+/CD4^+$: 285/mm^3	
	$CD3^+/CD8^+$: 76/mm^3		$CD3^+/CD8^+$: 2320/mm^3	
2	AHA, 1.3 y	Follow-up 36 mo		APDS
	IgG: 521 mg/dL		IgG: 1200* mg/dL	
	IgA: 44 mg/dL		IgA: 23 mg/dL	
	IgM: 92 mg/dL		IgM: 7 mg/dL	
	$CD19^+$: 295/mm^3		$CD19^+$: 306/mm^3	
	$CD3^+$: 2587/mm^3		$CD3^+$: 1453/mm^3	
	$CD3^+/CD4^+$: 1433/mm^3		$CD3^+/CD4^+$: 783/mm^3	
	$CD3^+/CD8^+$: 1013/mm^3		$CD3^+/CD8^+$: 545/mm^3	
			smB cells: 1.3%	
3	ITP, 10 y	Follow-up 48 mo		CVID
	IgG: 1360 mg/dL	Recurrent RTI	IgG: 701* mg/dL	
	IgA: 321 mg/dL	Pneumocystis pneumonia	IgA: 8 mg/dL	
	IgM: 67 mg/dL		IgM: 5 mg/dL	
	$CD19^+$: 269/mm^3		$CD19^+$: 337/mm^3	
	$CD3^+$: 1380/mm^3		$CD3^+$: 1526/mm^3	
	$CD3^+/CD4^+$: 515/mm^3		$CD3^+/CD4^+$: 550/mm^3	
	$CD3^+/CD8^+$: 718/mm^3		$CD3^+/CD8^+$: 930/mm^3	
			smB cells: 0%	
			Absent vaccine responses	
4	ITP, 7 y	Follow-up 48 mo		CVID
	IgG: 795 mg/dL	Recurrent RTI	IgG: 417* mg/dL	
	IgA: 73 mg/dL	Splenomegaly	IgA: <6 mg/dL	
	IgM: 106 mg/dL	Bronchiectasis	IgM: 5 mg/dL	
	$CD19^+$: 320/mm^3	Ground-glass changes at CT scan	$CD19^+$: 47/mm^3	
	$CD3^+$: 1275/mm^3		$CD3^+$: 1059/mm^3	
	$CD3^+/CD4^+$: 784/mm^3		$CD3^+/CD4^+$: 532/mm^3	
	$CD3^+/CD8^+$: 415/mm^3		$CD3^+/CD8^+$: 469/mm^3	
			smB cells: 0.7%	
			Transitional B cells: 14.6%	
			Absent vaccine responses	
5	AHA, 1.2 y	Follow-up 36 mo		PAD
	IgG: 432 mg/dL	Autoimmune hepatitis	IgG: 700* mg/dL	
	IgA: 36 mg/dL		IgA: <6 mg/dL	
	IgM: 43 mg/dL		IgM: <5 mg/dL	
	$CD19^+$: 356/mm^3		$CD19^+$: 0/mm^3	
	$CD3^+$: 2137/mm^3		$CD3^+$: 973/mm^3	
	$CD3^+/CD4^+$: 1276/mm^3		$CD3^+/CD4^+$: 712/mm^3	
	$CD3^+/CD8^+$: 861/mm^3		$CD3^+/CD8^+$: 171/mm^3	
6	AHA, 10 y	Follow-up 36 mo		PAD
	IgG: 1260 mg/dL	Recurrent RTI (Pseudomonas; *Staphylococcus aureus*)	IgG: 530* mg/dL	
	IgA: 44 mg/dL	Perianal abscess	IgA: 10 mg/dL	
	IgM: 87 mg/dL	(*Citrobacter freundii*; *Enterococcus gallinarum*)	IgM: 36 mg/dL	
	$CD19^+$: 114/mm^3	Salmonella spp infection	$CD19^+$: 138/mm^3	
	$CD3^+$: 430/mm^3	Lymphoproliferation	$CD3^+$: 1035/mm^3	
	$CD3^+/CD4^+$: 318/mm^3		$CD3^+/CD4^+$: 725/mm^3	
	$CD3^+/CD8^+$: 94/mm^3		$CD3^+/CD8^+$: 380/mm^3	

(continued)

TABLE VI. *(Continued)*

Patient no.	Pre-RTX phenotype	Post-RTX clinical phenotype	Post-RTX immune phenotype	Final diagnosis
7	ITP, 11.6 y	Follow-up 24 mo		CVID
	IgG: 772 mg/dL	Chronic rhinitisEBV chronic reactivation	IgG: 313* mg/dL	
	IgA: 15 mg/dL		IgA: <5 mg/dL	
	IgM: 56 mg/dL		IgM: 14 mg/dL	
	CD19+: ND		CD19+: 406/mm^3	
	CD3$^+$: ND		CD3$^+$: 1525/mm^3	
	CD3$^+$/CD4$^+$: ND		CD3$^+$/CD4$^+$: 684/mm^3	
	CD3$^+$/CD8$^+$: ND		CD3$^+$/CD8$^+$: 656/mm^3	
			Memory B cells: 3%	
			Transitional B cells: 15.7%	
			Plasma cells: 0.6%	
8	AHA, 14 y	Follow-up 48 mo		CVID
	IgG: ND	Recurrent RTIAnti-ADAMTS13 syndrome	IgG: 210* mg/dL	
	IgA: ND		IgA: <7 mg/dL	
	IgM: ND		IgM: <17 mg/dL	
	CD19+: ND		CD19+: 170/mm^3	
	CD3$^+$: ND		CD3$^+$: 1353/mm^3	
	CD3$^+$/CD4$^+$: ND		CD3$^+$/CD4$^+$: 493/mm^3	
	CD3$^+$/CD8$^+$: ND		CD3$^+$/CD8$^+$: 816/mm^3	
			smB cells: 0.5%	
9	AHA, 4.7 y	Follow-up 24 mo		CVID
	IgG: 843 mg/dL		IgG: 92* mg/dL	
	IgA: 114 mg/dL		IgA: 110 mg/dL	
	IgM: 91 mg/dL		IgM: 36 mg/dL	
	CD19+: 336/mm^3		CD19+: 207/mm^3	
	CD3$^+$: 1024/mm^3		CD3$^+$: 1752/mm^3	
	CD3$^+$/CD4$^+$: 400/mm^3		CD3$^+$/CD4$^+$: 873/mm^3	
	CD3$^+$/CD8$^+$: 528/mm^3		CD3$^+$/CD8$^+$: 712/mm^3	
			smB cells: 2%	
			Absent vaccine response	

APDS, Activated PI3K delta syndrome; *CMV*, cytomegalovirus; *CT*, computed tomography; *ND*, not determined; *RTI*, respiratory tract infection.
*Patients on immunoglobulin replacement therapy at last follow-up.

patients with CVID.[35] Interestingly, patients with PH had a high prevalence of impaired B-cell recovery (35%), which was already evident at 12 months post-RTX and preceded overt PH. Therefore, patients with a low B-cell count at 12 months but normal IgG level should be carefully monitored because PH may occur afterwards. However, even some patients with PH with normal B cells required long-term immunoglobulin replacement therapy. These children could present an intrinsic defect in smB-cell maturation, or hypogammaglobulinemia could be the result of an impaired cognate T_H-B-cell interaction. B-cell perturbation induced by RTX could unbalance T- and B-lymphocyte homeostasis, resulting in ineffective immunoglobulin production.

We also aimed to define a specific clinical phenotype that could help in identifying children at a higher risk of PH after RTX. Children with PH were significantly younger than patients with normal IgG levels. Even though the occurrence of more severe disease in children with PH cannot be excluded, another possible explanation is that autoimmune cytopenia could represent the first manifestation of an underlying immune impairment that was not fully discernible at the time of thrombocytopenia and/or hemolytic anemia onset. Conversely, in older children with autoimmune cytopenia, the presence of other clinical and/or immunologic features might facilitate the diagnosis of PID that *per se* could exclude them from the study. Pre-RTX IgG and lymphocyte subsets were not significantly different in patients with PH. However, comparison of absolute lymphocyte counts can be difficult because most of the patients were extensively treated with steroids and/or other immunosuppressive drugs. IgA and IgM levels were significantly lower in the PH group, probably reflecting younger age at diagnosis in this group, because patients with immunoglobulin levels less than 2 SD for age before treatment were equally distributed in the 2 groups. Moreover, some patients were treated with RTX at a very young age (<4 years), and it was not possible to discern children with potential transient hypogammaglobulinemia of infancy. However, our study mirrors a clinical practice experience, where unfortunately extensive immunologic workup is not always performed in children with refractory autoimmune cytopenia. Interestingly, we found a significantly higher risk of PH in patients with AHA or ES. Patients with PID present a higher incidence of autoimmune cytopenia, especially AHA and ES. Fischer et al[36] reported that more than 25% of PIDs have 1 or more autoimmune manifestations, and the risk of autoimmune cytopenia is estimated to be 120-fold higher than in the general population, indeed, up to 830-fold for autoimmune hemolytic

anemia in children with PIDs. This is consistent with our findings, because 9 patients with PH in our cohort were eventually diagnosed with a PID, supporting the hypothesis that PH after RTX is part of a PID phenotype. The higher rate of response to RTX in the PH group supports the hypothesis that B-cell depletion *per se* uncovers intrinsic immunologic defects. Indeed, constitutive immune system impairments were observed regardless of the cumulative exposure to anti-CD20 (total doses) or additional immunosuppression. However, autoimmune cytopenia also resolved in patients with PH who recovered B cells. Extensive analysis of B-cell subsets could help to clarify the mechanism of response to anti-CD20 mAb in children. Taken together, these observations suggest that patients who experience AHA or ES at a younger age and improvement/resolution of cytopenia after B-cell depletion need to be investigated extensively. Incomplete B-cell reconstitution at 12 months should encourage prolonged immunoglobulin level monitoring, B-cell subset analysis, and prompt clinical follow-up.

During follow-up, significant infectious and noninfectious complications were documented in the group of patients with PH, as part of complex clinical and immunologic phenotypes that are not usually reported in children treated with RTX. Specifically, a genetic diagnosis of 2 patients with PID and a clinical diagnosis of 7 patients with CVID or PAD were made. Seven of 9 patients did not present immunologic or clinical features before starting RTX, which could suggest an underlying immunologic defect. This could be attributed to the young age of the treated children, when immunologic defects were not easily discernible. Moreover, for all patients there was no suggestive history of PID before starting RTX, whereas infectious complications became relevant in the first years after treatment. This supports the hypothesis that B-cell depletion can alter immune system homeostasis of patients with a constitutive immunologic defect that was not appreciable before. However, because our data are partially retrospective, some limitations do not allow final conclusions and the impact of RTX on unveiling subclinical immunodeficiency needs to be carefully considered. Because the diagnosis of CVID and PAD was based on clinical criteria, it was not possible to exclude the potential iatrogenic effect of RTX to explain PH. However, the complex immunologic and clinical phenotype of these patients is unlikely due to RTX use in children with autoimmune cytopenia and can help to discern patients with PID from secondary immunodeficiency. Furthermore, although the additional effect of immunosuppressive treatment might contribute to delayed B-cell immune reconstitution, the only patient with PAD/CVID diagnosis who received cyclophosphamide, azathioprine, and cyclosporin before RTX never recovered B cells at 36 months after RTX, in the presence of normal T cells. Indeed, altered B-cell recovery is more likely due to an intrinsic B-cell defect, because anti-CD20 mAb cannot target early B-cell precursors that lack CD20 expression, rather than a direct prolonged effect of RTX or a combination of immunosuppressive treatments. A full and exhaustive immunologic workup, including immunoglobulins, lymphocyte subsets, and specific vaccine antibody responses, should be performed in all patients before starting RTX to discern the occurrence of new-onset hypogammaglobulinemia from preexisting immunologic defects and to evaluate other second-line treatments. During follow-up all immunoglobulin isotypes should be investigated and whenever patients develop immunoglobulin deficiency or other immunologic defects, extended analysis of lymphocyte subsets (ie, smB cells), vaccine responses, and genetic analysis are recommended for the early identification of those children presenting with autoimmune disease but with an underlying PID.

CONCLUSIONS

This is the largest cohort of children with autoimmune cytopenia with PH treated with RTX. We identified a specific subgroup of patients who should require a close and prolonged follow-up to achieve an early diagnosis of PID. These data should encourage a careful follow-up of these patients, at least until full immune reconstitution is achieved, and prospective studies should be encouraged to further explore the role of B-cell perturbation induced by RTX.

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