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ORIGINAL PAPER

Assessment of circulating blood lymphocytes in adult patients on rituximab to treat immune thrombocytopenia: Circulating number of NK cells is associated with the response at 6 months

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Summary

Immune thrombocytopenia (ITP) is defined by a low platelet count that can trigger potentially life-threatening haemorrhages. Three-quarters of adult patients exhibit persistent or chronic disease and require second-line treatments. Among these, rituximab, an anti-CD20 antibody, has yielded valuable results, with global responses in 60% of patients at 6 months and complete responses in 30% at 5 years. Factors predictive of response to ITP therapy would help physicians choose optimal treatments. We retrospectively analysed clinical courses, biological markers and blood lymphocyte subset numbers of 72 patients on rituximab to treat persistent/chronic ITP followed-up in our department between 2007 and 2021, divided into three groups according to the platelet count at 6 months: complete, partial or no response. Among all studied parameters, a low number of $CD3^{-}CD16^{+}CD56^{+}$ circulating NK cells was associated with the complete response to rituximab. We also found that, after rituximab therapy, complete responders exhibited increased NK and decreased activated $CD8^{+}$ T cell percentages. These results emphasize that the role played by NK cells in ITP remains incompletely known but that factors predictive of response to rituximab can be easily derived using blood lymphocyte subset data.

KEYWORDS
adult, immune thrombocytopenia, NK cells, rituximab

INTRODUCTION

Immune thrombocytopenia (ITP) is the most common form of autoimmune cytopenia worldwide and is associated with autoimmune platelet destruction and decreased platelet production.[1] Patients are thus exposed to potentially life-threatening bleeding episodes, particularly when the platelet count drops to below 20×10^9/L, or below 50×10^9/L when on concomitant antiplatelet or anticoagulant therapy.[2,3] Many treatments are being assessed and employed to raise the platelet count and stop severe bleeding. Among these, rituximab (RTX), a chimeric anti-CD20 antibody, has been extensively assessed and today serves as a second-line treatment for ITP; this is a splenectomy-sparing strategy.[3] In several previous studies, RTX induced global responses at 6 months and sustained complete responses at 5 years in approximately 60% and 30% of patients, respectively,[4,5] with a median time to response between 4 and 8 weeks.[6,7] RTX completely depletes B cells, creating a temporary, deep immune suppression for 6 to 12 months, increasing the infectious risk in patients who fail to respond to RTX and thus require subsequent splenectomy.[8] However, a recent meta-analysis that combined data

from five trials and long-term results of the ITP-ritux French registry showed that the infectious risk may not be as high as thought,[5,9] although the recent Covid-19 pandemic encouraged more cautious use of the drug in patients with autoimmune diseases.[10,11]

Factors predictive of a response to ITP therapy are urgently needed; many publications have addressed this problem, with mixed results.[5,7,12] Some monocentric retrospective studies and analyses of registries have suggested some predictive factors including disease duration <1 year, age < 40 years, the female sex and a previous transient response to steroids.[5,7,12] However, modifications of circulating lymphocyte subsets have not been considered in this context, perhaps because ITP pathophysiology is too heterogeneous. The decrease in platelet count reflects both antibody-mediated destruction of platelets and cytotoxic effects on platelets and megakaryocytes that involve T cells, B and plasma cells, macrophages and complement. Briefly, splenic, autoreactive T follicular helper cells initiate an autoimmune, antiplatelet antigen loop by inducing production of antiplatelet antibodies by B and plasma cells that further facilitate phagocytosis of opsonised platelets by macrophages in the reticuloendothelial system. These are the principal antigen-presenting cells that maintain the autoimmune loop over time. Autoreactive T cells are also involved in platelet destruction by increasing $CD8^+$ cell activation and cytotoxicity towards both circulating platelets and bone marrow megakaryocytes.[1,13–18]

In terms of the roles played by T cells in ITP, few studies have analysed the T cell subsets (or indeed the subsets of other lymphocytes) in peripheral blood of ITP patients,[19–22] particularly in the context of RTX treatment.[18,23] Thus, we retrospectively analysed data on peripheral blood lymphocyte subsets recorded for all ITP patients who received RTX and were followed-up in our centre.

METHODS

Patient recruitment

We retrospectively included patients with primary ITP on RTX followed-up at our centre from January 2007 to September 2021. All were at least 18 years of age and met the international consensus, primary ITP criteria.[24] Some evidenced only isolated biological autoimmunity, with no symptoms but were nonetheless considered to have primary ITP. All had failed first-line treatments with steroids and/or intravenous immunoglobulins (IVIGs). Additional exclusion criteria were thrombocytopenia not caused by primary ITP, a history of RTX treatment, prior splenectomy (because of the major role played by the spleen in ITP immune dysregulation), lost file data or no peripheral blood lymphocyte subset analysis prior to RTX, and/or concomitant treatment with a thrombopoietin-receptor agonist (TRA) that could interfere with the platelet response.

Definition of a response

Patients were divided into three groups by the platelet counts 6 months after RTX, as previously reported[24]: a complete response (CR) group with platelets $>100\times10^9$/L, a partial response (PR) group with platelets $30–100\times10^9$/L, and a no response (NR) group with platelets $<30\times10^9$/L and/or an indication for another treatment to raise the platelet count or cure bleeding.

Data collection

Patient data were anonymously extracted from medical files. We recorded age, sex, date of diagnosis, date of first RTX infusion, the date of the blood test that yielded the platelet count, platelet scintigraphy data (if any), previous treatments (steroids, IVIGs, disulone, hydroxychloroquine, TRAs) and the responses to those treatments, and the levels of antiplatelet, antinuclear and anti-phospholipid antibodies. All blood tests and labelled platelet scintigraphic tests were performed in departments of our hospital. The blood lymphocyte subsets were analysed as previously described[25] in the Bordeaux University Hospital Laboratory of Immunology employing a Beckman-Coulter FC500 flow cytometer. This yielded the total lymphocyte count and the percentages and numbers of $CD3^-$ (mainly B and NK cells), $CD19^+$ B cells, $CD3^-CD56^+CD16^+$ NK cells, $CD3^+$ T cells, $CD4^+$ T helper cells, $CD8^+$ cytotoxic T cells, $CD3^+CD4^-CD8^-$ cells, $CD3^+HLADR^+$ activated T cells, $CD3^+CD4^+HLADR^+$ activated T helper cells, and $CD3^+CD8^+HLADR^+$ activated cytotoxic T cells. Data were compared to those from a pool of 13 healthy controls.

Ethics

We performed a retroactive study on blood samples that were analysed during standard care in our unit; this research is in accordance with the French MR-004 methodology reference, and approved by our institutional review board. Patients were notified that their previously analysed blood samples will be reused for this study along with detailed information regarding the study itself, sent by mail and none refused to participate (non-opposition).

Statistics

Qualitative variables were grouped in terms of the response to RTX and compared using the chi-square test (significance level $p < 0.05$). We calculated the medians, quartiles (first and third) and minimal and maximal values of quantitative variables. Lymphocyte subset data before and after RTX (non-Gaussian pairs) were compared using the non-parametric Wilcoxon matched-pairs signed rank test (significance level $p < 0.05$). The distribution of the markers was compared across the three groups of responders using a Kruskal-Wallis test. Crude and adjusted p values (false discovery rate) for the

markers of interest (T cells and NK cells) are indicated. Only markers associated with the response with a crude p value <0.20 were included in a multinomial regression to evaluate their independent effect. Receiver operator curves were drown to determine sensitivities, specificities, and likelihood ratios, and for the relevant parameters, we chose the number with the best likelihood ratio + sensitivity + specificity. Statistical analyses employed GraphPad Prism ver. 6.0 (Graph Pad Software Inc, Dotmatics) and R software version 4.1.0 (R Project for Statistical Computing, RRID:SCR_001905).

RESULTS

Patients

From January 2007 to September 2021, 239 patients received RTX to treat ITP at Bordeaux Hospital, and we enrolled 72 in the current study (Figure 1). At least one measure on circulating lymphocyte status was required before the first RTX infusion. The median time between the first phenotyping and the first RTX infusion was 19 days (Table 1). Most patients were females (63%) and the median disease duration was 12 months, both factors being not statistically different between the three groups of response to treatment. At the time of first RTX infusion, all patients had received steroids (one-third continued on steroids during RTX therapy for 3 weeks) using the same protocol[3]; 75% received IVIGs (11% during RTX therapy). The median platelet count was 38×10^9/L. Half of all patients received RTX in two injections of 1 g, and the other half received RTX in four injections of 375 mg/m^2; both regimens yielded identical outcomes.[26] Of these very strictly selected patients, 31 (43%) experienced CRs at 6 months and 16 (22%) had PRs, yielding an overall response rate of 65%; 25 (35%) evidenced no response (NR patients). The patient characteristics by the ITP responses to RTX are shown in Table 1. The median ITP duration was higher in patients evidencing PRs to RTX (38 vs. 9 months for CR and 12 months for NR patients, $p = 0.006$). The high median ITP duration in the PR group is linked to the high heterogeneity of patients in this group with no clinical significance. More CR than PR or NR patients were on steroids at the time of RTX infusions ($p = 0.03$), but we could not find a difference according to whether patients experienced a transient response or not between groups. Moreover, age was not associated with the response ($p = 0.79$).

Finally, of the 31 patients experiencing CR at 6 months, 26 (84%) still evidenced CR at 24 months (the last CR patient received RTX in September 2019). Two patients received TPO-RAs at 9 and 18 months, one a single IVIG injection at 18 months (affording an immediate and persistent CR), and one disulone at 18 months; one was lost to follow-up after 6 months.

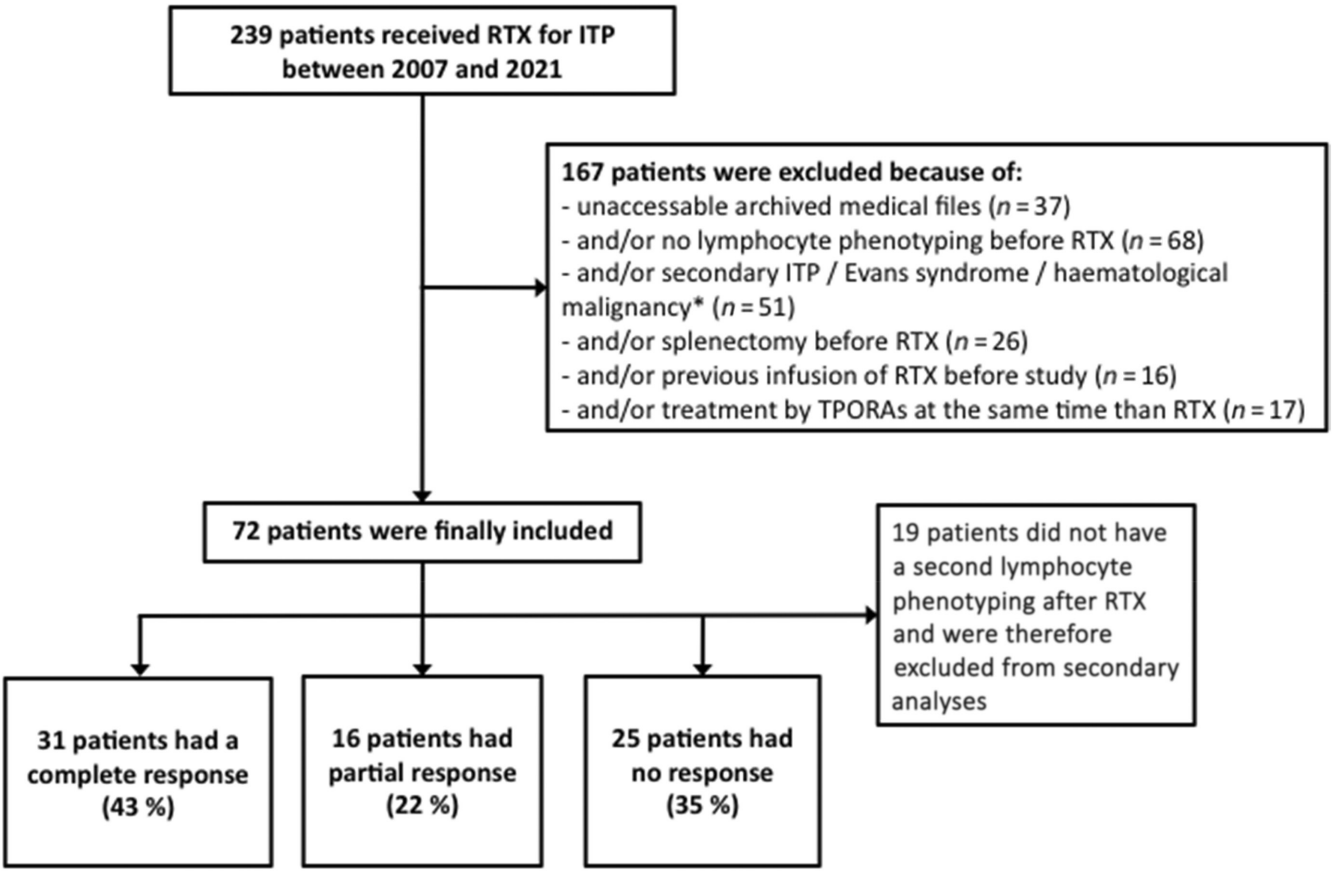

FIGURE 1 Flow chart of the study population. ITP, immune thrombocytopenia; RTX, rituximab; TPORAs, thrombopoietin-receptor agonists. Of note, excluded patients might have two or more associated exclusion criteria. *lymphoma, chronic lymphocytic leukaemia.

TABLE 1 General characteristics of the study population, by the response to rituximab.

	General	CR	PR	NR	*p*
N	72	31	16	25	
Median age (years, range)	52 (18–82)	51 (18–81)	47 (18–74)	58 (21–82)	0.79
Females (*n*, %)	45 (63)	21 (68)	12 (75)	12 (48)	0.16
Median ITP duration (months, range)	12 (1–288)	9 (1–156)	38 (5–288)	12 (1–180)	0.006
Median time between test and RTX (days, range)	19 (0–320)	17 (0–289)	17 (0–320)	19 (0–219)	0.95
Antiplatelet antibodies					0.32
Detected (*n*, %)	20 (28)	6 (19)	7 (44)	7 (28)	
Not detected (*n*, %)	29 (40)	15 (48)	7 (44)	7 (28)	
Missing data (*n*, %)	23 (32)	10 (32)	2 (12)	11 (44)	
Antinuclear antibodies					0.77
Positive (*n*, %)	22 (31)	11 (35)	4 (25)	7 (28)	
Negative (*n*, %)	46 (64)	19 (61)	11 (69)	16 (64)	
Missing data (*n*, %)	4 (6)	1 (4)	1 (6)	2 (8)	
Treatments before RTX					
Steroids	72 (100)				
Continuation of steroids while receiving RTX (*n*, %)	25 (35)	16 (52)	4 (25)	5 (20)	0.03
IVIG (*n*, %)	54 (75)	25 (81)	12 (75)	17 (68)	0.55
Disulone (*n*, %)	32 (44)	16 (52)	3 (19)	13 (52)	0.06
Hydroxychloroquin (*n*, %)	25 (35)	11 (35)	4 (25)	10 (40)	0.98
Median platelet count at day 1 ($\times 10^9$/L)[a]	38 (1–203)	60 (1–203)	41 (13–94)	38 (4–134)	0.46
Median platelet lifespan (days, range)	3.9 (1.3–8)	3.5 (2–7)	4.5 (2.5–8)	3.75 (1.3–6)	0.57
Splenic sequestration (*n*, %)	34 (50)	11 (35)	7 (44)	16 (64)	0.24
Hepatic platelet sequestration (*n*, %)	13 (18)	5 (16)	2 (12)	6 (24)	
Missing data (*n*, %)	25 (35)	15 (48)	7 (44)	3 (12)	
RTX dose and schedule					0.58
1 g on days 1 and 15 (*n*, %)	36 (50)	14 (45)	9 (56)	13 (52)	
375 mg/m^2 on days 1, 8, 15 and 22 (*n*, %)	36 (50)	17 (55)	7 (44)	12 (48)	
Median time between the two lymphocyte phenotypings, months	12 (4–31)	14.5 (4–24)	11 (4–21)	15 (4–31)	0.39
Missing data (no second phenotypings, *n*, %)	19 (26)	6 (19)	4 (25)	9 (36)	

Abbreviations: CR, complete response; ITP, immune thrombocytopenia; IVIGs, intravenous immunoglobulins; NR, no response; PR, partial response; RTX, rituximab.
[a]Some patients previously received steroids or IVIG.

Lymphocyte subsets in ITP patients compared to controls

We first compared the lymphocyte subsets of ITP patients to those of controls before and after RTX treatment (Table 2). Before RTX, ITP patients had a lower lymphocyte count (median 1574 vs. 2112/mm^3, $p=0.04$) and lower numbers of T cells (1174 vs. 1653/mm^3, $p=0.08$), T CD8$^+$ cytotoxic T cells (319 vs. 509/mm^3, $p=0.02$), and CD3$^+$CD4$^-$CD8$^-$ cells (48 vs. 82/mm^3, $p=0.01$), than controls. These figures did not change markedly after RTX therapy. The lymphocyte number remained low (median 1496/mm^3, $p=0.007$), notably that of CD3$^-$ cells (263 vs. 466/mm^3, $p=0.002$) (principally CD19$^+$ B cells [40 vs. 191/mm^3, $p=0.0006$]), as would be expected after CD20$^+$ cell-depletion therapy. However, T cells numbers remained low (1163/mm^3, $p=0.01$), mainly T CD4$^+$ helper cells (717/mm^3, $p=0.03$) and CD3$^+$CD4$^-$CD8$^-$ cells (49/mm^3, $p=0.04$). Notably, the lymphocyte subset percentages were generally similar to those of controls, suggesting that lymphopenia was homogeneous in ITP patients.

Factors associated with the treatment response among the lymphocyte subset data

When comparing the lymphocyte subsets at baseline before starting RTX, we found no differences between groups for most parameters, especially CD4$^+$ or CD8$^+$ T cells, or CD4/CD8 ratio (Figure 2A–C). However, we found that, in a smaller subset of patients with available measures,

TABLE 2 Lymphocyte subset data of ITP patients and controls before and after rituximab treatment.

	Before RTX					After RTX (median time 12 months)				
Response to RTX	**CR, *n* = 31**	**PR, *n* = 16**	**NR, *n* = 25**	**CTL, *n* = 13**	***p*- value**	**CR, *n* = 25**	**PR, *n* = 12**	**NR, *n* = 16**	**CTL, *n* = 13**	***p*- value**
Total circulating lymphocytes, cells/mm^3	1514	1950	1416	2112	0.04	1442	1447	1605	2112	0.007
(B & NK cells) CD3$^-$, cells/mm^3	332	466	343	466	0.10	253	282	295	466	0.002
% lymphocytes	23.8	23.8	25.6	24	0.98	20.5	17.92	19.34	24	0.51
(B cells) CD19$^+$, cells/mm^3	160	238	249	191	0.38	58	15	31	191	0.0006
% lymphocytes	14.9	12.82	17.27	9.16	0.08	3.58	2.60	3.39	9.16	0.004
(T cells precursors) CD3$^-$CD4$^-$CD8$^-$, cells/mm^3	212	308	239	378	0.17	234	178	267	378	0.002
% lymphocytes	21.33	16.51	20.79	18.58	0.79	16.11	12.65	16.83	18.58	0.38
(NK cells) CD3$^-$CD16$^+$CD56$^+$, cells/mm^3	80	225	157	224	0.005	159	207	175	224	0.45
% lymphocytes	6.93	11.25	9.47	9.28	0.27	13.13	11.48	9.49	9.28	0.61
(T cells) CD3$^+$, cells/mm^3	1068	1398	1120	1653	0.08	1110	1282	1277	1653	0.01
% lymphocytes	75.73	73.9	74.36	76.02	0.91	79.96	83.29	80.68	76.02	0.47
(T helper cells) CD3$^+$CD4$^+$, cells/mm^3	764	785	736	1040	0.29	614	749	780	1040	0.03
% lymphocytes	44.59	43.37	49.20	45.39	0.35	47.73	49.71	52.57	45.39	0.74
(Cytotoxic T cells) CD3$^+$CD8$^+$, cells/mm^3	338	399	269	509	0.02	357	388	345	509	0.17
% lymphocytes	22.93	23.62	20.21	25.07	0.33	25.1	28.9	32.77	25.07	0.52
CD3$^+$CD4$^-$CD8$^-$, cells/mm^3	41	78	52	82	0.01	51	51	49	82	0.04
% lymphocytes	3.17	3.72	3.22	3.56	0.55	3.49	3.89	3.21	3.56	0.51
(Activated T cells) CD3$^+$HLADR$^+$, % of CD3$^+$ lymphocytes	9.59	7.33	8.52	7.14	0.36	6.9	11.13	7	7.14	0.42
(Activated T helper cells) CD3$^+$CD4$^+$HLADR$^+$, % of CD3$^+$ lymphocytes	7.78	7.12	6.8	6.48	0.67	8	9.52	6.9	6.48	0.24
(Activated cytotoxic T cells) CD3$^+$CD8$^+$HLADR$^+$, % of CD3$^+$ lymphocytes	23.76	17.84	24.48	14.89	0.25	19	21.56	17	14.89	0.68

Note: The data are medians. The more green the colour, the greater the increase compared to the controls; the more orange the colour, the greater the decrease.

Abbreviations: %, percentage; *N*, number; RTX, rituximab.

$CD3^-CD16^+CD56^+$ circulating NK cells were lower in CR as compared to the other groups (median 80/mm^3 vs. 225/mm^3 for PR patients and 157/mm^3 for NR patients, $p = 0.0087$, adjusted p value = 0.04, Figure 2D). A threshold ratio of 150/mm^3 afforded 83% specificity and 60% sensitivity in terms of response prediction (likelihood ratio 3.4). We tested the independent predictive effect of $CD4^+$, $CD8^+$, CD4/CD8 ratio and NK cells in a multinomial model, based on the significant results found between ITP patients and controls before RTX as shown in Table 2. We found that only a higher NK cells count was associated with an increased probability of being partial responder (OR = 5.92 for 100 cells higher, $p = 0.01$) or non-responder (OR = 3.42 for 100 cells higher, $p = 0.05$) as compared to complete responder. Therefore, a circulating NK cell level under 150/mm^3 could predict RTX response in ITP patients.

Lymphocyte subsets before and after RTX

We secondarily analysed how the lymphocyte subsets changed before and after RTX by the type of response (Figure 2E,F). A median time of 12 months separated the two phenotypings in 53 patients (74%). The delay between RTX and the second phenotyping (<12 or ≥12 months) only influenced the results in the <12-month group for two

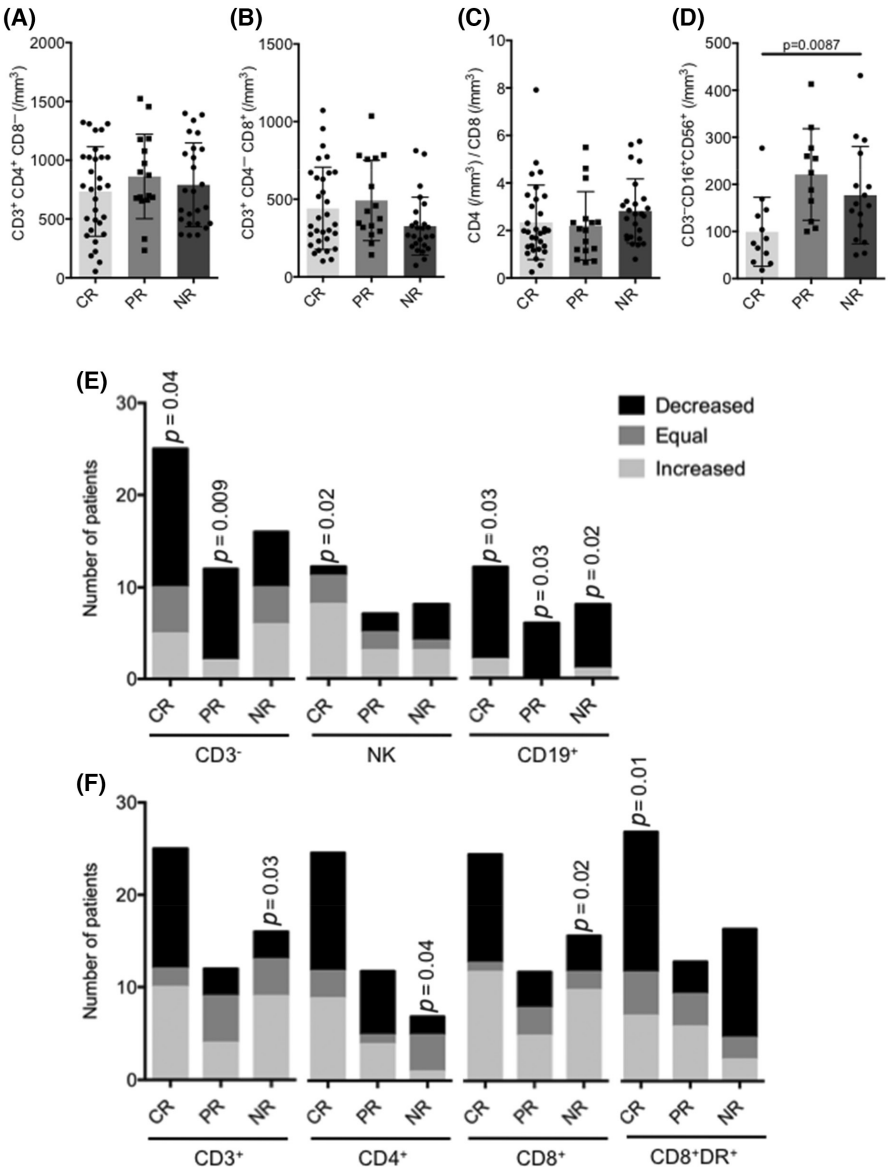

FIGURE 2 Lymphocyte subsets in the blood of ITP patients. (AD) The number of circulating $CD4^+$ T cells (A), $CD8^+$ T cells (B), the CD4/CD8 ratio (C) and the number of circulating NK cells (D) by the RTX response. (EF) Evolution of the circulating numbers of $CD3^-$, $CD19^+$, NK cells (E) and $CD3^+$, $CD4^+$, $CD8^+$ cells with the percentage of activated T $CD8^+$ cells (F) before and after rituximab. For each parameter, the lymphocyte subset data before and after RTX in each patient were compared using the non-parametric Wilcoxon matched-pairs signed rank test. Changes > +10% (increase) or < −10% (decrease) were considered significant, otherwise the two parameters for each patient were considered equal. CR, complete response; NR, no response; PR, partial response.

parameters: the $CD3^-$ cells count in PR patients ($p = 0.04$) and the percentages of $CD3^+CD8^+HLADR^+$ activated cytotoxic T cells in the CR group ($p = 0.007$) (Figure S1). As expected, $CD3^-$ lymphocyte numbers decreased significantly after RTX, particularly those of $CD19^+$ B cells, most notably in PR patients. Interestingly, we noticed significant increases in $CD3^+$, $CD4^+$ and $CD8^+$ T cell numbers in non-responders compared to PR and CR patients. Moreover, CR patients evidenced decreased percentages of $CD3^+CD8^+HLADR^+$ activated cytotoxic T cells and increased numbers of $CD3^-CD56^+CD16^+$ NK cells. The CD4/CD8 ratios did not differ significantly before and after RTX (median 1.9, 2 and 2.4 in CR, PR and NR patients, respectively) because of a decrease in the ratio in NR patients (from 2.69 to 2.4). We found no variation of the effect of RTX on the change of the markers according to the groups (CR/PR).

DISCUSSION

In a selected population of 72 non-splenectomised patients who received RTX between 2007 and 2021 to treat primary ITP for whom peripheral blood lymphocyte phenotyping data were available prior to RTX, we found that a low number of circulating NK cells (<150/mm^3) could predict the response to RTX. In contrast to previous observations,[5,7,12] age, sex, duration of disease and the previous response to steroids did not predict the RTX response, possibly because of our strict selection process. The overall response to RTX at 6 months was 65%, in line with those of previous reports,[4–7] and notably, 84% of CR patients still evidenced CR at 24 months.

We also analysed the peripheral blood lymphocyte subsets after RTX, especially more than 6 months after therapy, to explore the persistent reorganization of the immune system after B cells reappeared. We sought new insights into ITP pathophysiology. This analysis was possible for 75% of patients with a median of 12 months (range 4–31 months) between the two lymphocyte-phenotyping tests before and after RTX, this median time only very mildly impacted the results.

All patients evidenced homogeneous lymphopenia, compared to the controls. Interestingly, all ITP patients also had lower numbers of T cells (either $CD4^+$ T helper cells, $CD8^+$ cytotoxic T cells or doubly negative $CD3^+CD4^-CD8^-$ T cells) but not B cells. In the ITP context, few have explored the circulating numbers of peripheral lymphocytes. An increase in the B cell percentage was found in one study, explained principally by elevation of naïve B cell numbers; we noted only a trend in elevation of the B cell percentage compared to that of controls ($p = 0.08$).[27] This lymphocyte subset is important in the ITP context given the major role played by B cells in ITP pathophysiology. B cells produce antiplatelet auto-antibodies, present platelet antigens to T cells, and secrete cytokines (the BAFF/BAFFR dysregulation).[28] ITP patients also evidenced lower numbers of circulating $CD8^+$ cytotoxic T cells than controls, unlike what was previously described. Li et al. found a higher percentage of circulating $CD8^+$ T cells in 15 untreated ITP patients than 13 controls but the absolute cell counts were not given.[14] Audia et al. reported equivalent percentages of $CD8^+$ T cells in 30 ITP and eight controls[29] but a significant increase in the Th1 cell percentages of eight ITP patients responding to RTX but not in 10 non-responders. These are the opposite of our findings, although we did not engage in interferon-labelling of $CD3^+$ cells. Interestingly, as suggested by Audia et al., we found a decrease in the percentage of activated cytotoxic T cells in responders, but an increase in the number of circulating cytotoxic T cells in non-responders. The decrease in responders may indicate less T cell-mediated destruction of platelets, particularly in the spleen, where the microenvironment favours close contacts among cells,[29] or in bone marrow where T-cells impair platelet production by destroying megakaryocytes.[14,15] The effects of B-cell-depleting therapies such as RTX on T cells have been explored in non-responding ITP patients who were consequently splenectomised, and the results have suggested that RTX increases the spleen Th1/Treg ratio compared to that of patients who do not receive RTX.[30] Moreover, we previously described periarteriolar T-cell-zone expansion of the spleen of patients who failed to respond to RTX before splenectomy.[31] Together, the findings confirm the major role played by cytotoxic T cells in terms of platelet destruction, mostly in the spleen; this correlates indirectly with the numbers of circulating $CD8^+$ T cells in RTX non-responders.

Finally, we found that the number of circulating NK cells was initially low in CR patients, but increased significantly once a response was attained. Previous studies have reported contrasting results. Three studies involving 30[22] and 35[19] children and 14 NR adults[27] found that the percentages decreased; this is the opposite of what we noted. Two other studies reported similar numbers of circulating NK cells,[21,32] and one study found an increase in the number of CD3-$CD56^+$ NK cells (purified from $CD2^+$ cells) in 23 patients with therapy-dependent disease.[33] The role played by NK cells in ITP pathophysiology is becoming clearer; such cells secrete IFN-γ that induces a Th1 response.[34] The cells are involved in ADCC-mediated platelet destruction, which is increased by RTX in ITP patients,[35] mainly in the spleen.[2,11] The NK capacities of cells decrease.[21,32] We advance two hypotheses about the role played by T and NK cells in ITP pathophysiology. First, the T and NK cells of responders might evidence lower cytotoxicity towards platelets, leading to a lower "pressure in the autoimmune destruction of platelets" that is further reduced when RTX interrupts the dialogue between B and T cells. Second, non-responders who evidence lower numbers of NK cells before RTX might have NK cells that are defective in terms of the capacity to suppress autoimmune responses, as has been previously shown in other autoimmune diseases. NK cells can control autoantigen release and the adaptive immune response, particularly by killing immature dendritic cells presenting autoantigens, and by activating macrophages.[36] It is possible that RTX restored

this suppressive capacity of NK cells in responders. This hypothesis deserves further exploration in a study on NK cell subpopulations.

Our study had both strengths and limitations. This was a retrospective, quantitative, monocentric study; we did not perform functional analyses of circulating T cells and patients were very strictly selected. Moreover, validation of the NK predictive parameter must be made on a larger cohort given the low sensitivity and negative predictive values. However, for many patients on RTX, we suggest that this factors easily derived from routine blood analysis predict the response. Together, the data suggest that this factor should be assessed in a larger population of ITP patients, or in a randomized controlled trial, to determine if it better identifies ITP patients who should receive RTX or other second-line treatments such as disulone, mycophenolate mofetil, a TRA or fostamatinib.

AUTHOR CONTRIBUTIONS

Etienne Rivière, Rodolphe Thiébaut, Estibaliz Lazaro, Alexandre Guy, Chloé James, Olivier Mansier, Patrick Blanco and Jean-François Viallard were responsible for conceptualization, methodology, writing—original draft, writing—review & editing, supervision and project administration.

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CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

All data can be available upon request to the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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