TO THE EDITOR:

Low-dose rituximab in autoimmune hemolytic anemia: 10 years after

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Rituximab is becoming the preferred second-line choice for steroid-refractory warm autoimmune hemolytic anemia (wAIHA) and the first-line choice for cold agglutinin disease (CAD). However, rituximab is an expensive treatment that is not available worldwide; it is also not in indication or reimbursable in all countries. It is usually administered at 375 mg/m^2 once weekly for 4 weeks, with high efficacy (overall response rate [ORR] >80%) in wAIHA, both idiopathic and secondary forms.[1-5] At variance, cold forms exhibit lower response rates (40%-50%, mainly partial) when rituximab is used as a single agent; therefore, combination treatments with purine analogues (eg, fludarabine, bendamustine) have been proposed with better ORRs, although with side effects.[6] Even if rituximab has a good safety profile, infusion reactions, immunosuppression, and hepatitis virus/mycobacterial reactivations can occur. To minimize side effects and reduce costs, and considering the lower lymphocyte burden in autoimmune hematologic conditions compared with lymphoproliferative diseases, low-dose (LD) rituximab (100 mg fixed dose once weekly for 4 weeks) has been used in several autoimmune diseases, including immune thrombocytopenic purpura and AIHA.[7-10]

Ten years ago, we prospectively assessed the efficacy of LD rituximab in primary wAIHA and CAD in a pilot study (#NCT01345708); we reported ~80% response rates in wAIHA and ~60% in CAD, along with a ~50% reduction in steroid administration.[10] Here, we continued to prospectively evaluate 20 of the original patients and included an additional 34 consecutive patients from June 2012 until April 2018, according to the original protocol (100 mg fixed dose once weekly for 4 weeks combined with a short course of prednisone, starting at 1 mg/kg per day with subsequent tapering and discontinuation within 3 months). As shown in Table 1, wAIHA cases accounted for one-half of the patients, followed by CAD, mixed, and atypical cases. All patients enrolled had hemoglobin levels <10 g/dL or lactate dehydrogenase levels >1.5 × the upper limit of normal, and the great majority had received at least 1 course of steroids. The time from diagnosis of AIHA to initiation of rituximab therapy was ≥12 months in 15 cases (3 of whom had received cytotoxic immune suppressors and exhibited slightly reduced immunoglobulin levels at baseline). Patients were followed up for a median of 53 months (range, 6-120 months), and ORR was invariably >80% within the first 3 years; complete response (CR) rates increased from 46% at month +2 to >60% at month +6 and thereafter.

Because recent evidence pinpoints hemoglobin levels at onset and AIHA type as predictors of relapse/refractoriness to therapy,[11,12] we investigated the efficacy of LD rituximab in the various AIHA forms, divided into wAIHA, CAD, mixed (direct antiglobulin test result positive for immunoglobulin G [IgG] plus high-titer C), and atypical ones (negative direct antiglobulin test result). Response rates were better in wAIHA compared with other forms (CAD,

Table 1. Clinical and laboratory characteristics of patients with AIHA at enrollment and treatment outcome

Characteristic	Patients (N = 54)
Age, median (range), y	66 (20-90)
Sex, n (%)	
Female	32 (59)
Male	22 (41)
AIHA type, n (%)	
wAIHA IgG	23 (42.6)
wAIHA IgG+C	4 (7.4)
Cold	20 (37)
Mixed	4 (7.4)
Atypical	3 (5.6)
Hemoglobin, median (range), g/dL	9.6 (4.4-13.2)
LDH × ULN, median (range)	1.36 (0.5-4.5)
Unconjugated bilirubin, median (range), mg/dL	1.72 (0.4-9.7)
Reticulocytes, median (range), ×10^9/L	145 (27-550)
IgA, median (range), g/L	137 (42-385)
IgG, median (range), g/L	681 (401-1014)
IgM, median (range), g/L	110 (32-303)
Time to LD rituximab, median (range), mo	7 (0-89)
Prospective follow-up, median (range), mo	53 (6-120)
Month +2 ORR/CR, n (%)	46 (85)/25 (46)
Month +6 ORR/CR, n (%)	35 (83)/26 (62)
Month +12 ORR/CR, n (%)	30 (88)/23 (68)
Month +18 ORR/CR, n (%)	20 (91)/15 (68)
Month +24 ORR/CR, n (%)	18 (95)/15 (79)
Month +36 ORR/CR, n (%)	8 (89)/6 (67)
Duration of response, median (range), mo	15 (3-85)
RFS, median (range), mo	41.9 (14.3-69.5)
Relapsed, n (%)	28 (62)

wAIHA direct antiglobulin test result positive for IgG only (wAIHA IgG) and wAIHA direct antiglobulin test result positive for IgG and low-titer C (wAIHA IgG+C) types included 14 cases from the pilot study and an additional 13 cases; CAD included 9 and 11 patients, respectively. Four mixed and 7 atypical AIHA cases were all newly treated in this study.
LDH, lactate dehydrogenase; ULN, upper limit of normal.

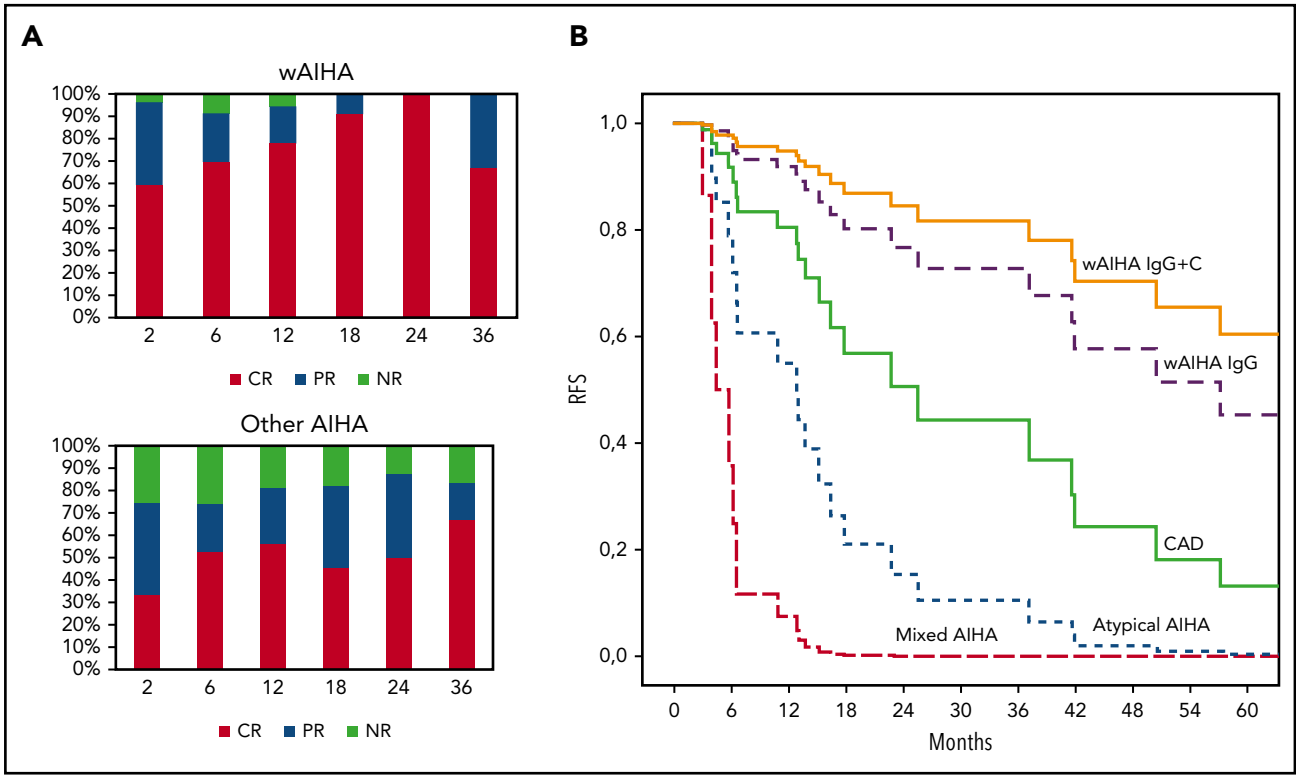

Figure 1. Response rate and RFS according to AIHA type. (A) Response rates in wAIHA and in all other forms (time cutoff, month +36). (B) RFS according to Cox regression model (timeline cutoff, month +60; n = 25). NR, no response; PR, partial response; wAIHA IgG, wAIHA direct antiglobulin test result positive for IgG only; wAIHA IgG+C, wAIHA direct antiglobulin test result positive for IgG and low-titer C.

mixed, and atypical; $P = .05$). In particular, in the former, ORR was >90% at each time point (Figure 1A), and CR rates ranged from 59% (month +2) to 100% (month +24). This amelioration of CR over time is in line with the immune-modulating effect of rituximab that may emerge beyond the well-known B-cell–depleting activity, as already shown on cytokine levels in the pilot study.[10] Moreover, the progressive increase in response rates further suggests that rituximab takes a while to work, although the number of evaluable patients decreases over time. No relationship was found among response rates and baseline hematologic parameters, or with the time from diagnosis to initiation of rituximab. Response rates were comparable considering patients enrolled in the pilot study and the new patients. We observed no grade 3 or 4 adverse events, and IgA, IgG, and IgM serum levels at month +12 were comparable to baseline (mean values of 167 ± 133 mg/dL, 758 ± 385 mg/dL, and 153 ± 162 mg/dL, respectively). Median duration of response was 1 year and 3 months, and 62% of patients relapsed. Relapsed patients received various treatments, including steroids (n = 15), cytotoxic immune suppressors (3 azathioprine, 2 cyclophosphamide), splenectomy (5), bortezomib (3), and rituximab at standard doses (3); 14 cases were retreated with LD rituximab, of whom 9 (64%) with further response. Relapse-free survival (RFS) significantly correlated with AIHA type: it was longer in wAIHA (both IgG and IgG+low-titer C) compared with other cases (64 months [95% CI, 26.6-102] vs 25 months [95% CI, 9.4-41.6]; $P = .004$]) and was particularly short in mixed and atypical cases (Figure 1B). Notably, the depth of response at 1 year was also related to longer RFS, and hemoglobin levels at 12 months positively correlated with duration of response ($r = 0.37$; $P = .02$). In multivariable analysis according to Cox regression models, the presence of wAIHA emerged as the only significant predictor of longer RFS ($P = .01$). Concerning long-term outcomes, 25 cases reached the 5-year follow-up, and 5 of them (20.5%) are long-term responders after the first LD rituximab course.

The analysis of these 10 years of prospective data confirms the efficacy of LD rituximab in primary AIHA, both as a short-term response as well as a long-term outcome. Most cases responded within the first 2 months, and rituximab's immune-modulating activity continued in responders, with further amelioration of the response after month 6. We clearly showed that LD rituximab has better efficacy and induces sustained responses in warm cases compared with CAD; this finding supports the hypothesis that patients with CAD would benefit from a higher rituximab dose because of a greater burden of clonal autoreactive B cells.[6] Moreover, the depth of response correlated with longer response duration, similar to what has been observed in onco-hematologic diseases. In terms of safety, we confirmed that LD rituximab is safe and tolerable, with no significant changes in baseline immunoglobulin levels in the 1 year since treatment; LD rituximab can also be safely re-administered, with an efficacy in up to 60% of cases and with a cumulative dose lower than that reached with 1 course at standard doses. These findings suggest that retreatment with LD rituximab seems more appropriate to gain a fine-tuning of the autoimmune reactivity, at variance with the massive B-cell depletion obtained with standard doses. Our study was not designed to compare low vs standard rituximab doses in terms of efficacy, safety, and immune reconstitution,

however, and an ad hoc study would be advisable to address these issues. Regarding response rates, our data compare well with those reported in several studies and meta-analysis for standard doses.[1,2,4,12,13] Likewise, RFS seems comparable between the 2 schedules, with 20.5% long-term responders at 5 years in the present cohort and 20% in a retrospective multicentric study.[12] Finally, a lower dose (about one-seventh of the standard dose) may help in reducing costs for the health services.

Altogether, our data advise the use of LD rituximab as early second-line treatment in wAIHA, particularly in the presence of reactivation of hemolysis during initial steroid tapering, in patients at risk for steroidal side effects or in frail/elderly cases.[14] At variance, this schedule seems less effective in cold, mixed, and atypical cases, suggesting the use of standard doses in these settings.

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