REGULAR ARTICLE

blood advances

Recombinant erythropoietin in autoimmune hemolytic anemia with inadequate bone marrow response: a prospective analysis

Bruno Fattizzo,[1,2] Giacinto Luca Pedone,[1,2] Caterina Brambilla,[1,2] Loredana Pettine,[1] Anna Zaninoni,[1] Francesco Passamonti,[1,2] and Wilma Barcellini[1]

[1]Hematology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; and [2]Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy

Key Points

- Recombinant erythropoietin induced an Hb response >50% at 15 days and 70% at 1 month in AIHA with inadequate bone marrow compensation.
- Treatment was effective independently by AIHA subtype and safe without increased risk of thrombosis.

Up to 30% of patients with autoimmune hemolytic anemia (AIHA) show inadequate bone marrow (BM) compensatory response with inappropriately low levels of reticulocytes and endogenous erythropoietin. Ineffective BM compensation is associated with more severe anemia, transfusion need, and hospital admission, and treatment with recombinant erythropoietin (rEPO) may be beneficial. Here, we prospectively analyzed the efficacy and safety of rEPO in a single-center cohort of 47 patients with AIHA with inadequate reticulocytosis and endogenous erythropoietin at baseline. Epoetin alpha 40 000 international units per week were administered subcutaneously until hemoglobin (Hb) >11 g/dL and then tapered off. Overall response was 55% at 15 days, 74% at 1 month, 74% at 3 months, 80% at 6 months, and 91% at 12 months. Consistently, Hb values significantly increased from baseline to each subsequent time point (P<.001) with a median increase of +1.4, +2.4, +3.4, +3.8, and +4.4 g/dL, respectively. Transfusion needs reduced from 30% to <10% at 15 days and thereafter (P < .001). Concomitant medications included prednisone or methylprednisolone (N = 40, stable since >2 weeks from enrollment), mycophenolate mofetil (N = 1, ongoing since >3 months from enrollment), and rituximab (N = 7 patients with cold agglutinin disease from day 8). No association between concomitant medications and response to rEPO was found. Treatment was generally safe without rEPO-related severe adverse events. The comparison with an AIHA population not treated with rEPO showed a significant benefit of rEPO at 15 days and 1 month on response and Hb increase. These data support the use of rEPO as an add on to standard immunosuppression in AIHA with inadequate BM compensation. This trial was registered at www.clinicaltrials.gov as #NCT05931718.

Introduction

Autoimmune hemolytic anemia (AIHA) is a rare disease characterized by immune mediated destruction of erythrocytes.[1] AIHA is classified according to the isotype and thermal features of the autoantibody in warm (wAIHA), cold (cold agglutinin disease [CAD]), mixed, and atypical forms; the disease is further termed primary or secondary according to the presence of concomitant conditions.[1,2] The clinical

Submitted 26 September 2023; accepted 17 November 2023; prepublished online on *Blood Advances* First Edition 29 November 2023; final version published online 12 March 2024. https://doi.org/10.1182/bloodadvances.2023011798.

All data are available within the article, and further data may be available upon reasonable request to the corresponding author, Bruno Fattizzo (bruno.fattizzo@unimi.it).

The full-text version of this article contains a data supplement.

presentation is highly heterogeneous, from compensated to rapidly worsening, and treatment is based on immunosuppressive drugs, particularly steroids and rituximab, along with transfusion support.[2] The role of bone marrow (BM) compensation has recently emerged as an important determinant of AIHA prognosis. It has been reported that 1 of 3 patients may have reticulocyte levels inappropriately low for hemoglobin (Hb) values; these subjects showed more severe anemia and transfusion need than those with adequate reticulocytosis.[3-5] Furthermore, a recent multicenter retrospective analysis of patients with AIHA showed the benefit of boosting erythropoiesis by recombinant erythropoietin (rEPO), particularly in subjects with inadequate reticulocyte counts.[6] With the aim of confirming these results in a prospective study, we evaluated the safety and efficacy of epoetin α in patients with AIHA with inadequate reticulocytosis and inappropriate levels of endogenous erythropoietin.

Materials and methods

Patient population

Forty-seven patients with primary AIHA followed at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano were consecutively enrolled from January 2019 until December 2022.

The study was approved by the local ethical committee as a substudy of the CYTOPAN observational trial (NCT05931718) and was conducted according to the Declaration of Helsinki.

Inclusion criteria were (1) AIHA diagnosis as per current recommendations,[2] (2) Hb <10 g/dL with lactate dehydrogenase (LDH) >1.5 × upper limit of normality (ULN), and (3) inadequate reticulocytosis (BM responsiveness index [BMRI] <121) and inappropriate levels of endogenous erythropoietin.[6] BMRI was calculated as (patient absolute reticulocytes × Hb/normal Hb)/1000. Appropriateness of endogenous EPO per Hb levels was determined according to previous publications as (log[EPO] = 4.478 − [0.284 × Hb]).[6]

Exclusion criteria were presence of nutrient deficiency, bleeding, kidney failure (ie, creatinine >1.5 × ULN or glomerular filtration rate <50 mL/min), liver enzyme alteration (>1.5 × ULN), and inability of signing an informed consent. Additional exclusion criteria were treatment with steroids (prednisone or methylprednisolone), if not on stable dose since at least 2 weeks, rituximab given <3 months before enrollment, IV immunoglobulins (IVIG) <3 weeks before enrollment, and cytotoxic immunosuppressants (cyclosporine, cyclophosphamide, mycophenolate mofetil, and azathioprine), if not on stable dose since at least 3 weeks. Concomitant treatments were allowed on clinical basis and included transfusions, steroid increase, IVIG, and rituximab (from day 8), and were systematically recorded. Patients were stratified according to the type of direct antiglobulin test (DAT) positivity as wAIHA (IgG or IgG plus complement [C]), CAD (DAT positive for C and cold agglutinin titer ≥64 at 4°C), mixed (IgG and C and high titer cold agglutinins), and atypical forms (IgA and DAT-negative). Hematologic parameters at diagnosis and at enrollment, along with previous AIHA therapies, were collected at baseline.

Treatment with recombinant EPO and outcomes

Patients were treated with epoetin α at the flat dose of 40 000 international units (IUs) subcutaneously per week. The dose was selected based on previous retrospective experience,[6] as well as on the dose used for myelodysplastic neoplasms whose marrow features may be similar to that of active AIHA with inadequate reticulocytosis. The first injection was delivered at the hospital and the next at home. Hematologic parameters (Hb g/dL, LDH U/L, and reticulocytes × 10^9/L) and number of red blood cell units transfused were evaluated at baseline, at 15 days, and at 1, 3, 6, and 12 months. Response was defined complete response for Hb ≥ 12 g/dL with normalization of hemolytic parameters, and partial response for Hb ≥ 10 g/dL or Hb increase from baseline of at least 2 g/dL without transfusions. Relapses were defined as decrease in level of Hb <10 g/dL or at least 2 g/dL from the previous visit, along with evidence of worsening hemolysis. Adverse events were systematically registered according to the Common Terminology Criteria for Adverse Events, version 5.0.

For patients reaching Hb >11 g/dL, rEPO was tapered to 40 000 IU every 14 days and stopped if Hb >12 g/dL or in case of drug-related adverse events.

Comparison with an AIHA population not treated with rEPO

Data from patients diagnosed with AIHA within the period between January 2019 and December 2022, showing a BMRI < 121 and inadequate endogenous EPO but not receiving rEPO as per physician or patient choice, were systematically recorded within the observational CYTOPAN protocol. This population served as a comparison for the study population (ie, those treated with rEPO) but not as a formal "control cohort" because of the lack of randomization.

Statistical analysis

Descriptive statistics were used to define mean, median, standard error, and ranges of each continuous variable. Student *t* test or Wilcoxon test were used for continuous variables. For categorical variables, we used χ^2 test or Fisher exact test when >20% of observations had expected frequencies of <5.

Results

Demographic and hematologic features at baseline

A total of 47 patients were enrolled (Table 1). Median age was 69 years (range, 19-94) and 29 patients (62%) were older adults (ie, >60 years), with a male to female ratio of 1.13. Forty-seven percent of the patients had a warm type, 40% a CAD, and 13% a mixed or atypical form; 6 patients had a concomitant immune neutropenia or thrombocytopenia, namely Evans syndrome.

At enrollment, 57% had severe anemia (ie, Hb values <8 g/dL), without significant differences among AIHA categories; median reticulocytes and endogenous EPO were 121 × 10^9/L (range, 10-266) and 55.9 U/L (range, 10-457), respectively, inadequate in all patients as per inclusion criteria. Particularly, median endogenous EPO was ~20% of that expected for Hb levels (ie, ~300 U/L for Hb 7.5 g/dL). BM trephine biopsy showed hypercellularity in 60% of cases, dyserythropoiesis in 79%, and reticulin fibrosis (grade 1 according to the World Health Organization) in 41%, along with a polyclonal lymphoid infiltrate in almost all subjects (mainly T cell, median 5% of total cellularity; range, 3%-12%).

Table 1. Clinical and hematologic features of patients with AIHA at enrollment

	All (N = 47)	wAIHA (N = 22)	CAD (N = 19)	Mixed/atypical (N = 6)
Distribution %	100%	47%	40%	13%
Median age, y (range)	69 (19-94)	71 (19-89)	66 (33-94)	80 (25-89)
Males/females, N	25/22	12/10	9/10	4/2
Hematologic parameters				
Hb (g/dL)	7.6 (3.2-10)	7.4 (5-10)	7.6 (5.8-10)	8.2 (3.2-9.7)
Hb < 8 g/dL, n (%)	27 (57%)	13 (59%)	11 (58%)	3 (50%)
Ht (%)	22.7 (8.4-33)	23 (13.8-33)	23 (17-30)	21 (8.4-28.4)
LDH (U/L)	374 (153-2083)	422 (195-2083)	341 (153-1073)	293 (228-430)
Ret ($\times 10^9$/L)	121 (10-266)	106 (42-266)	130 (44-191)	136 (10-204)
BMRI	76 (2.7-120)	83 (2.7-117)	73 (26.8-119.8)	77 (17.5-120)
EPO (U/L)	56 (10-457)	51 (10.8-230)	62 (17-457)	91 (10-327)
BM features				
Cellularity, %	48 (10-95)	45 (10-80)	43 (18-80)	75 (40-95)
Hypercellularity, n (%)	26 (60)	10 (56)	11 (58)	5 (83)
Dyserythropoiesis, n (%)	34 (79)	14 (78)	15 (79%)	5 (83)
Fibrosis, n (%)	17 (41)	7 (39)	8 (47)	2 (33)
Lymphoid infiltrate, n (%)	37 (90)	14 (88)	17 (89)	6 (100)
Lymphoid infiltrate, %	5% (1-13)	7.5% (2.5-13)	7% (2.5-12)	5% (1-5)

Atypical AIHA were 2 DAT-negative patients. Values are given as median (range) unless otherwise specified.
Ht, hematocrit; Ret, reticulocytes; BMRI, bone marrow responsiveness index; BM, bone marrow; EPO, endogenous erythropoietin.

Clinical data at diagnosis and AIHA medical history are summarized in supplemental Tables 1 and 2. In brief, 40% of patients required red blood cell transfusions (median, 2 units per patient; range, 1-6), and 91% received a previous AIHA medication (median, 2 lines; range, 0-6), including steroids (87%), rituximab (47%), IVIG (25%), and cytotoxic immunosuppressors (36%, azathioprine, cyclosporine, or cyclophosphamide). Only 1 patient had undergone splenectomy.

Treatment and response evaluation

Median time from AIHA diagnosis to rEPO was 15 months (range, 0-34), with 9 patients (19%) being treated upfront. As shown in Figure 1, overall response rate (ORR, complete response + partial response) was 55% at day 15, 74% at month 1, 74% at month 3, 80% at month 6, and 91% at month 12. Consistently, Hb values significantly increased from baseline to each subsequent time point ($P < .001$). Median increase was +1.4, +2.4, +3.4, +3.8, and +4.4 g/dL at the various time points. Transfusion needs progressively reduced to 9%, 6%, 4%, 7%, and 0% at day 15, month 1, 3, 6, and 12, respectively ($P < .001$). Notably, Hb values were obtained before each transfusion episode.

At day 15, ORR and Hb values were higher in patients with wAIHA than those with CAD (73% vs 26%, $P = .004$; and 11.3 [range, 72-147] vs 9.7 g/dL [range, 49-129], $P = .03$), irrespective of Hb values at baseline, and were equal at the subsequent time points (supplemental Figure 1). LDH progressively decreased (273 [range, 167-1657] at 15 days vs 374 U/L [range, 153-2083] at baseline; $P = .02$), whereas reticulocytes increased at 15 days (184×10^9/L [range, 28-510], $P < .001$ vs baseline) and then progressively decreased.

In total, 12 patients relapsed (26%, 9 wAIHA, 1 CAD, and 2 mixed), at 3 months (N = 5), 6 months (N = 5), and 1 year (N = 2) of follow-up.

Concomitant medications

At enrollment, 94% of patients were receiving a concomitant medication, mainly prednisone or methylprednisolone (N = 40, 85%, 20 wAIHA, 14 CAD, 4 mixed, 2 atypical) at a median dose of 0.5 mg/kg per day (range, 0.01-1), stable since at least 2 weeks. Seven patients (19%, 6 wAIHA, 1 mixed) had received IVIG at least 3 weeks before, and 1 patient with wAIHA had mycophenolate mofetil ongoing since at least 3 months. During the study, 7 CAD subjects received 4 weekly doses of rituximab 375 mg/sm from day 8. No association between concomitant medications and response to rEPO was found. Responses were observed in patients with CAD irrespective of rituximab treatment (supplemental Figure 2).

Treatment duration and safety

Median treatment duration was 4 months (range, 1 week-4 years), and 5 patients (19%) received a single dose of epoetin α (3 CAD and 2 wAIHA), with subsequent discontinuation owing to rapid Hb increase. At 1 year, 35 patients (74%) had stopped rEPO mainly because of persistent response (64%). Other reasons included nonresponse (4%), adverse events (1%, grade 3 pulmonary embolism during admission for pneumonia and active hemolysis), and death in 2 (4%) older patients with wAIHA (1 heart attack and 1 metastatic colorectal carcinoma).

A total of 5 adverse events were registered: 2 thrombosis (the abovementioned pulmonary embolism, and 1 deep venous thrombosis of the lower limb in the patient with active wAIHA who had undergone splenectomy); 1 acute renal failure during active

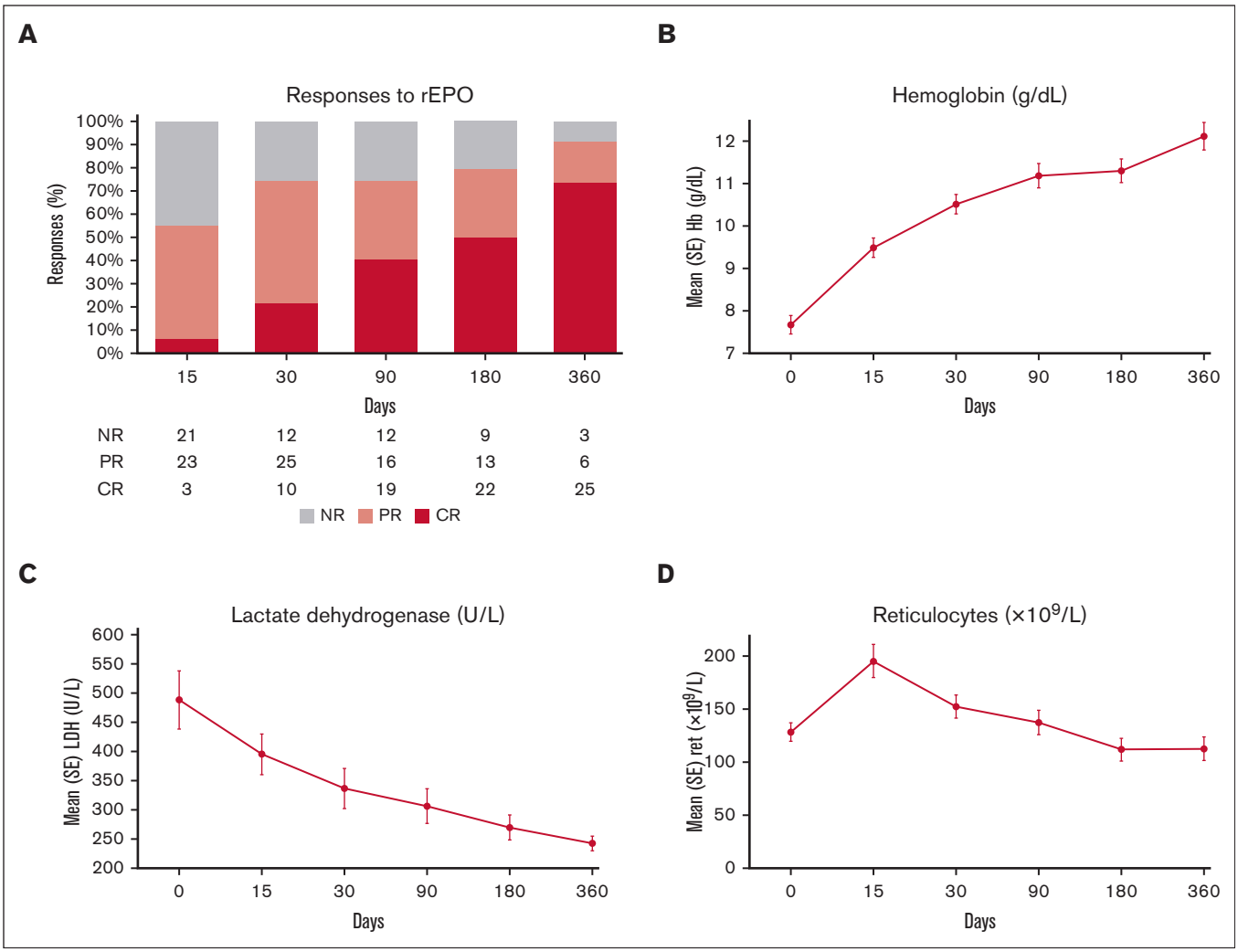

Figure 1. Efficacy of rEPO in patients with AIHA. (A) Response rates (complete response/partial response); (B) Hb levels; (C) LDH; and (D) reticulocytes values along the follow-up. Values are given as mean and standard errors. CR, complete response; PR, partial response; NR, no response; SE standard error.

wAIHA; and 2 infections (grade 3 *Legionella* spp pneumonia in a CAD, and *Listeria monocytogenes* sepsis in a wAIHA).

Comparison with an AIHA population not treated with rEPO

Forty-three patients with AIHA with BMRI < 121 and inadequate endogenous EPO treated at our center in the same period did not receive rEPO as per physician or patient choice. Demographic and hematologic features and previous AIHA therapies were comparable with the rEPO cohort (supplemental Tables 3 and 4, $P > .05$). Specifically, median Hb at baseline was 8.1 g/dL (range, 4.2-10.1), BMRI 86 (range, 3-121), and endogenous EPO 106 U/L (range, 36-200). Endogenous EPO was higher than that in patients treated with rEPO, but with largely overlapping ranges, and inadequate in all cases. AIHA medications during observation were similar between patients treated with rEPO and those not treated with rEPO (steroids 88%, rituximab 19%, IVIG 12%, traditional immunosuppressors 14%; $P > .05$ for all). As shown in Table 2, ORR was higher in the rEPO cohort than in the non-rEPO cohort at all time points, reaching statistical significance at day 15 (55% vs 32%, odds ratio [OR] 2.39; 95% confidence interval, 1.00-5.67; $P = .04$). Consistently, Hb values were significantly higher in the former at day 15 (9.4 g/dL [range, 5.8-12.3] vs 8.6 g/dL [range, 4.4-12.2]; $P = .04$), at month 1 (10.5 g/dL [range, 4.9-13.7] vs 9.5 [range, 6.4-13.9]; $P = .05$), and 1 year (12.2 g/dL [range, 8.8-15.1] vs 11.2 g/L [range, 7.3-17]; $P = .03$). Even transfusion need at day 15 was lower in patients treated with rEPO than in those not treated with rEPO (9% vs 15%), although not significantly. The frequency of adverse events was the same in the 2 cohorts, with 2 thromboses, 2 infections, and 1 acute renal failure in patients not receiving rEPO. With regard to mortality, 1 patient died of progression to T-cell lymphoma in the non-rEPO cohort.

Discussion

This prospective study demonstrates the efficacy of rEPO as an add on to standard immunosuppression in a prospective cohort of 47 patients with AIHA with inadequate BM compensatory response,

Table 2. Response rates and hematologic values in patients receiving rEPO vs the comparison population

	rEPO (N = 47)	No rEPO (N = 43)
Baseline		
Hb g/dL, mean (SD)	7.6 (1.7)	7.8 (1.4)
Transfusions (%)	14 (30)	15 (35)
15 d	47	43
ORR (%) - CR/PR*	26 (55) - 3/23	14 (32) - 1/3
Hb g/dL, mean (SD)*	9.5 (1.5)	8.8 (1.5)
Transfusions (%)	4 (9)	7 (15)
1 mo	47	43
ORR (%) - CR/PR	35 (74) - 10/25	25 (58) - 4/21
Hb g/dL, mean (SD)*	10.5 (1.9)	9.8 (1.5)
Transfusions (%)	3 (6)	4 (9)
3 mo	47	43
ORR (%) - CR/PR	35 (74) - 19/16	30 (69)- 12/18
Hb g/dL, mean (SD)	11.2 (1.9)	10.8 (1.9)
Transfusions (%)	2 (4)	1 (2)
6 mo	44	42
ORR (%) - CR/PR	35 (80) - 22/13	30 (71) - 13/17
Hb g/dL, mean (SD)	11.3 (2.1)	10.8 (1.8)
Transfusions (%)	3 (7)	2 (5)
12 mo	34	39
ORR (%) - CR/PR	31 (91) - 25/6	29 (74) - 13/16
Hb g/dL, mean (SD)	12.1 (1.4)	11.1 (2)
Transfusions (%)	0 (0)	1 (3)

CR, complete response; PR, partial response; SD, standard deviation.
*$P < .05$.

independently from previous therapy lines or concomitant medication.

The prospective analysis carries the additional value of selecting a homogenous AIHA population as per serologic subtype distribution, diagnosis of primary AIHA, and baseline reticulocyte and endogenous EPO levels.

Importantly, a rapid median increase of 1.4 g/dL of Hb was already evident at 15 days and progressively increased along time. This early response was associated with a reduction of transfusion need from 30% to 9%. This may contribute to reduce the risk of alloimmunization, reported to be as high as 30% in large retrospective series.[1,2,7,8] Moreover, addition of rEPO to the traditional immunosuppressive approach may be useful to fasten the response, particularly to rituximab that may require even months to be effective.[3,9,10] Finally, rEPO addition may be particularly beneficial in the setting of severe acute AIHA, marked by high rate of complications and admission to an intensive care unit. A recent French study reported a mortality as high as 12.9% in AIHA admitted to intensive care unit, at a median of 3.5 days of admission. Interestingly, 92% of patients had inadequate reticulocytosis, and only 22.6% of them had received rEPO.[11]

The use of rEPO in AIHA may change the treatment paradigm of such "increased turn over anemia" and allows the reduction of the immunosuppressive burden in this patient population, at higher risk of infections, a main cause of morbidity and mortality.[3,4]

The inadequate BM response in AIHA has been related to the presence of anti-erythroblast autoantibodies,[5,12,13] to a "shocked" BM incapable of reacting during the acute phase, or to the presence of an underlying hematologic or infectious disease.[6,14] In the European retrospective analysis, ineffective erythropoiesis in AIHA was associated with an altered cytokine profile suggesting a role of BM microenvironment in this setting.[6] The relatively high prevalence of CAD in the study population (40%) may indicate that inadequate BM response is a prevalent feature in patients with CAD who are generally older and comorbid as compared with those with wAIHA; the nature of our tertiary center being the referral for difficult-to-treat AIHA forms, including CAD, may be a second reason.

Treatment with rEPO was safe as documented by a similar rate of adverse events in patients with AIHA receiving the drug vs a comparison group not treated with rEPO, although with the caveat of a nonrandomized trial. Thrombotic events, a potential life-threatening complication,[1-3] were observed in only 2 cases with active AIHA and concomitant risk factors (ie, active infection and previous splenectomy). This frequency is consistent with previous reports disclosing a 10% to 20% rate of thrombosis in AIHA, particularly in patients with LDH > 1.5 × ULN and concomitant risk factors.[2,15] One could postulate that the same AIHA population deserving rEPO may also benefit from low molecular weight heparin prophylaxis thus further reducing the risk.[15]

Notably, most patients interrupted rEPO after a median time of 2 months, as in persistent response. For clinical practice, this indicates that a use "on demand" is more frequent as compared with a continuous therapy. From a biological perspective, this feature is consistent with a "transitory ineffective erythropoiesis" in AIHA vs BM failures.

Our study carries several limitations because of the relatively small number of patients and the lack of a randomized controlled cohort. We decided to perform an open label study because some prior evidence of effectiveness and safety was already available in the literature[2,6,16]; however, we included the AIHA population not treated with rEPO as per physician or patient choice, enrolled in the prospective CYTOPAN protocol, to allow some comparison. In addition, the contribution of concomitant medication to response cannot be excluded, although the inclusion of patients unresponsive or with inadequate response to stable doses of steroids or cytotoxic immunosuppressants may overcome the bias. Rituximab was administered from day 8 of rEPO in 7 patients with CAD, but hematologic response did not differ between patients receiving and those not receiving the drug. Notwithstanding the limited numbers, it may be hypothesized that the effect of rEPO is mainly evident within the first month from rEPO start, thus not affecting the comparison of rEPO efficacy in the 2 subgroups of patients. Moreover, the comparison with a cohort of AIHA not treated with rEPO in the same time strengthened the early benefit of rEPO treatment. In any case, the effect of concomitant immunosuppression remains essential for establishing AIHA response (particularly amelioration of hemolytic markers), so that we propose rEPO as an add-on treatment.

In conclusion, this prospective study supports the use of rEPO on top of immunosuppressive therapy to obtain an early erythroid response and to reduce transfusion need in AIHA with inadequate BM compensation.

Acknowledgments

The study was partially funded by Italian Ministry of Health, Current Research Grant.

Authorship

Contribution: B.F., G.L.P, C.B., L.P., and W.B. followed patients, designed the study, collected data, wrote the article, and revised the manuscript for important intellectual content; A.Z. collected data and revised the manuscript for important intellectual content; F.P. revised the manuscript for important intellectual content; and all authors approved the present submission.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: B.F., 0000-0003-0857-8379; G.L.P., 0009-0000-7727-2766; L.P., 0000-0001-9553-2082.

Correspondence: Bruno Fattizzo, Hematology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, via Francesco Sforza 35, 20100 Milan, Italy; email: bruno.fattizzo@unimi.it.

References

1. Berentsen S, Barcellini W. Autoimmune hemolytic anemias. *N Engl J Med*. 2021;385(15):1407-1419.
2. Jäger U, Barcellini W, Broome CM, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: recommendations from the First International Consensus Meeting. *Blood Rev*. 2020;41:100648.
3. Barcellini W, Fattizzo B, Zaninoni A, et al. Clinical heterogeneity and predictors of outcome in primary autoimmune hemolytic anemia: a GIMEMA study of 308 patients. *Blood*. 2014;124(19):2930-2936.
4. Barcellini W, Zaninoni A, Fattizzo B, et al. Predictors of refractoriness to therapy and healthcare resource utilization in 378 patients with primary autoimmune hemolytic anemia from eight Italian reference centers. *Am J Hematol*. 2018;93(9):E243-E246.
5. Fattizzo B, Zaninoni A, Gianelli U, et al. Prognostic impact of bone marrow fibrosis and dyserythropoiesis in autoimmune hemolytic anemia. *Am J Hematol*. 2018;93(4):E88-E91.
6. Fattizzo B, Michel M, Zaninoni A, et al. Efficacy of recombinant erythropoietin in autoimmune hemolytic anemia: a multicenter international study. *Haematologica*. 2021;106(2):622-625.
7. Hendrickson JE, Tormey CA. Understanding red blood cell alloimmunization triggers. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):446-451.
8. Petz LD. "Least incompatible" units for transfusion in autoimmune hemolytic anemia: should we eliminate this meaningless term? A commentary for clinicians and transfusion medicine professionals. *Transfusion*. 2003;43(11):1503-1507.
9. Berentsen S, Barcellini W, D'Sa S, et al. Cold agglutinin disease revisited: a multinational, observational study of 232 patients. *Blood*. 2020;136(4):480-488.
10. Berentsen S, Ulvestad E, Gjertsen BT, et al. Rituximab for primary chronic cold agglutinin disease: a prospective study of 37 courses of therapy in 27 patients. *Blood*. 2004;103(8):2925-2928.
11. Pouchelon C, Lafont C, Lafarge A, et al. Characteristics and outcome of adults with severe autoimmune hemolytic anemia admitted to the intensive care unit: results from a large French observational study. *Am J Hematol*. 2022;97(10):E371-E373.
12. Barcellini W. New insights in the pathogenesis of autoimmune hemolytic anemia. *Transfus Med Hemother*. 2015;42(5):287-293.
13. Zaninoni A, Imperiali FG, Cattaneo A, et al. Detection of erythroblast antibodies in mitogen-stimulated bone marrow cultures from patients with myelodysplastic syndromes. *Transfusion*. 2016;56(8):2037-2041.
14. Cho JN, Avera S, Iyamu K. Pancytopenia as a consequence of sepsis and intravenous antibiotic drug toxicity. *Cureus*. 2019;11(2):e3994.
15. Fattizzo B, Bortolotti M, Giannotta JA, Zaninoni A, Consonni D, Barcellini W. Intravascular hemolysis and multitreatment predict thrombosis in patients with autoimmune hemolytic anemia. *J Thromb Haemost*. 2022;20(8):1852-1858.
16. Salama A, Hartnack D, Lindemann HW, Lange HJ, Rummel M, Loew A. The effect of erythropoiesis-stimulating agents in patients with therapy-refractory autoimmune hemolytic anemia. *Transfus Med Hemother*. 2014;41(6):462-468.