How do we treat life-threatening anemia in a Jehovah's Witness patient?

Joseph A. Posluszny Jr and Lena M. Napolitano

The refusal of allogeneic human blood and blood products by Jehovah's Witness (JW) patients complicates the treatment of life-threatening anemia. For JW patients, when hemoglobin (Hb) levels decrease beyond traditional transfusion thresholds (<7 g/dL), alternative methods to allogeneic blood transfusion can be utilized to augment erythropoiesis and restore endogenous Hb levels. The use of erythropoietin-stimulating agents and intravenous iron has been shown to restore red blood cell and Hb levels in JW patients, although these effects may be significantly delayed. When JW patients have evidence of life-threatening anemia (Hb <5 g/dL), oxygen-carrying capacity can be supplemented with the administration of Hb-based oxygen carriers (HBOCs). Although HBOCs are not Food and Drug Administration (FDA) approved, they may be obtained and administered with FDA, institutional review board, and patient approval. We describe a protocol-based algorithm to the management of life-threatening anemia in JW patients and review time to anemia reversal and patient outcomes using this approach.

The management of Jehovah's Witness (JW) patients with anemia and bleeding presents a clinical dilemma as they do not accept allogeneic human blood or blood product transfusions.[1,2] With increased understanding of the JW patient beliefs and blood product limitations, the medical community can better prepare for optimal treatment of severe life-threatening anemia in JW patients.

Lower hemoglobin (Hb) is associated with increased mortality risk in JW patients. In a study of 300 patients who refused blood transfusion, for every 1 g/dL decrease in Hb below 8 g/dL, the odds of death increased 2.5-fold (Fig. 1).[3] A more recent single-center update of JW patients (n = 293) who declined blood transfusion reported an overall mortality rate of 8.2%, with a twofold increased risk of death per each 1 g/dL decrease in nadir Hb (unadjusted odds ratio [OR], 1.04; 95% confidence interval [CI], 1.52-2.74; adjusted OR, 1.82; 95% CI, 1.27-2.59; Fig. 1).[4]

A variety of treatment strategies have been used to improve the efficiency and production of red blood cells (RBC) and to limit blood loss in JW patients, including erythropoiesis-stimulating agents (ESAs), iron

ABBREVIATIONS: AMRS = Anemia Mortality Risk Score; ESA(s) = erythropoiesis-stimulating agent(s); HBOC(s) = hemoglobin-based oxygen carrier(s); IND = investigational new drug; JW = Jehovah's Witness; MI = myocardial infarction.

From the Division of Acute Care Surgery [Trauma, Burns, Critical Care, Emergency Surgery], Department of Surgery, University of Michigan, Ann Arbor, Michigan.

Address reprint requests to: Lena M. Napolitano MD, FACS, FCCP, FCCM, Department of Surgery, University of Michigan Health System, Room 1C340-UH, University Hospital, 1500 East Medical Drive, SPC 5033, Ann Arbor, MI 48109-5033; e-mail: lenan@umich.edu.

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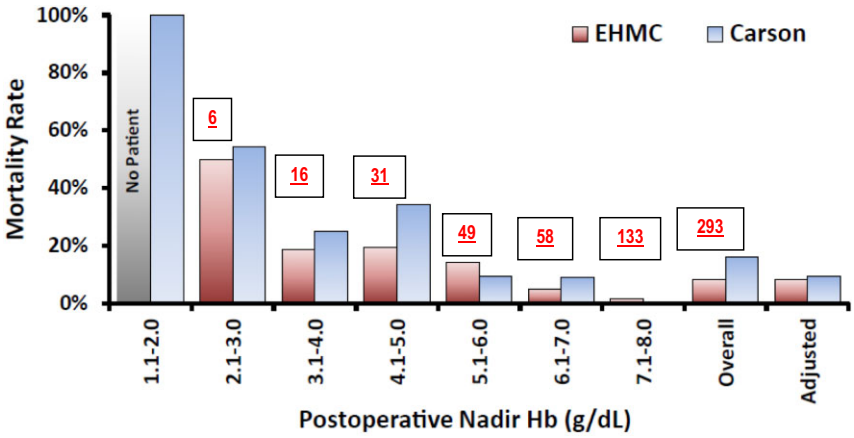

Fig. 1. Mortality rates in patients (n = 293) who decline blood transfusion stratified by nadir postoperative Hb concentrations in two published studies. The last pair of bars represent the data from Carson and colleagues[3] adjusted according to the number of EHMC patients in each nadir Hb category. EHMC cohort had no patient with nadir Hb level of less than 2 g/dL. Numbers at the top reflect "n" in each group. EHMC = Englewood Hospital and Medical Center. From Shander A, Javidroozi M, Naqvi S et al. An update on mortality and morbidity in patients with very low postoperative Hb levels who decline blood transfusion. Transfusion. 2014 Feb 17. [Epub ahead of print][4] Carson = Carson JL, Noveck H, Berlin JA et al. Mortality and morbidity in patients with very low postoperative Hb levels who decline blood transfusion. Transfusion 2002;42:812-8.

supplementation, Hb-based oxygen carriers (HBOCs), and blood conservation strategies.[5] The ultimate goal is to preserve oxygen-carrying capacity in all ways possible without allogeneic human blood transfusion. We developed a standardized treatment algorithm (Fig. 2) for management of severe anemia in JW patients, using all available treatments for severe life-threatening anemia.

DEFINITION OF SEVERE ANEMIA AND MORTALITY RISK

There is no universally accepted definition of severe anemia. Based on the current literature, which includes case reports, case series, and retrospective analyses, we define severe anemia as Hb of less than 5 or 5 to 7 g/dL with symptoms of hypoperfusion including lactic acidosis, base deficit, shock, hemodynamic instability, or coronary ischemia. Retrospective studies have confirmed that mortality rates significantly increase with Hb concentrations between 5 and 6 g/dL in patients who refuse allogeneic transfusion.[6] We initiate our anemia protocol in all JW patients with a Hb level of less than 7 g/dL, but we consider the use of HBOCs in patients with a Hb level of less than 5 to 6 g/dL with examination of the individual patient's anemia tolerance and adequacy of oxygen delivery including assessment of adequacy of perfusion, cardiac complications, and lactate or base deficit.

In JW patients, two additional studies have shown that other patient factors in addition to anemia are risk factors for mortality. The additional risk factors identified by the Auckland Anemia Mortality Risk Score (Auckland AMRS) include age of at least 45 years of age, weight of at least 90 kg, hypertension, cardiac arrhythmia, angina, previous myocardial infarction (MI), valvular heart disease, heart failure, being on hemodialysis, acute admission, and Hb level of not more than 8 g/dL on admission to the hospital. A composite score of these risk factors stratifies mortality rates of 4% to 83% (AMRS 0-3, 4% mortality; AMRS 4-5, 32% mortality; AMRS 6-7, 50% mortality; AMRS ≥ 8, 83% mortality).[7]

The Hamilton AMRS further refined their risk model by including treatment-related mortality risk factors during hospitalization (shock, acute gastrointestinal bleeding, pneumonia, nadir Hb ≤ 7 g/dL, sepsis, worse congestive heart failure, neurologic complications [stroke and hypoxic encephalopathy]). Higher Hamilton AMRS scores were associated with high mortality (0-2, 4% mortality; 3-4, 29%; 5, 40%; ≥6, 67%).[8] Although these risk assessment scores have not yet been validated in large multicenter studies, they promote the fact that there are high mortality rates associated with anemia in JW patients.

TREATMENT STRATEGIES FOR SEVERE ANEMIA

A multimodal approach is commonly required for treatment of JW patients with severe anemia, including treatments to enhance endogenous erythropoiesis, reduce blood loss, increase oxygen delivery, reduce oxygen consumption, and avoid hemodilution and iatrogenic anemia.

ESAs

Exogenous ESAs are an effective anemia therapy by inducing proliferation and terminal differentiation of endogenous erythroid cells and prevention of erythroid cell apoptosis in the marrow.[9] ESAs also exert a potent protective effect against hypoxia by induction and activation of hypoxia inducible factor-1α with antiapoptotic action.[10] ESAs may also affect renal tubular function leading to a decrease in plasma volume and a relative increase in Hb levels.[11] RBC expansion with ESA therapy is evidenced by an increase in reticulocyte count by Day 3 of

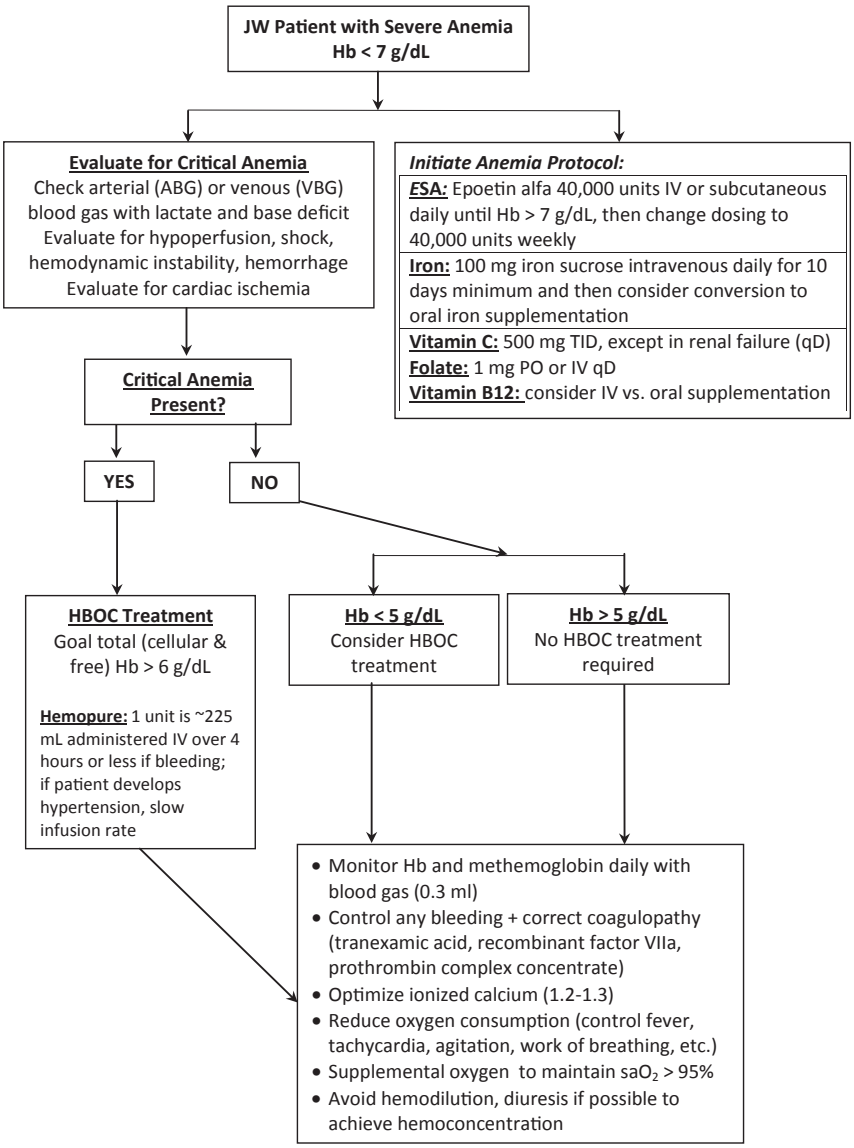

Fig. 2. Severe anemia protocol for JW patients.

treatment in patients who are iron replete, with the equivalent of 1 blood unit produced by Day 7 of ESA treatment.[12] As a result, the effects of ESAs on overall RBC number or Hb level may not be present for several days after initial administration. ESA therapy, despite its delayed effect, is a valuable tool for JW patients for whom allogeneic blood transfusion is not an option.

ESAs are not recommended for anemia treatment in critically ill patients. A meta-analysis of nine RCTs confirmed that ESA use in critically ill patients was associated with a decreased number of transfused patients (OR, 0.73; 95% CI, 0.64-0.84; $I^2 = 54.7\%$) and a small reduction in the mean number of units (0.41 unit) transfused per patient (95% CI, 0.10-0.74; $I^2 = 79.2\%$)[13] and another study documented potential increase in venous thromboembolism.[14] However, in the severely anemic JW patient, any increase in erythropoiesis via ESAs may be lifesaving. It has, however, been documented that high pharmacologic ESA doses can overcome the erythropoietin (EPO) resistance seen in the anemia of inflammation which is common in critically ill[15] and severe anemia patients.[16]

The optimal ESA dose for treatment of JW patients with severe anemia is unknown. A randomized trial of six different ESA dosing regimens in critically ill patients determined that all were well tolerated and appeared to effect reticulocytosis with a peak on Day 11 or Day 15. Interestingly, the pharmacokinetics did not predict the pharmacodynamics response in anemic critically ill patients.[17] Our protocol uses 40,000 units of EPO IV of subcutaneous daily until the Hb level is more than 7 g/dL. Furthermore, adequacy of iron supplementation was in question in this study.[18] This is an important issue, as maximal erythropoietic response will not be achieved with ESAs if relative iron deficiency occurs. Iron-deficient erythropoiesis was documented in critically ill patients treated with ESAs and intravenous (IV) iron with confirmation of elevated zinc protoporphyrin concentrations.[19] No specific studies have yet examined the optimal ESA dose in JW patients with severe anemia.

Iron supplementation

Adequate iron stores are necessary to ensure adequate erythropoiesis.[20] Similar to ESAs, the effect of iron on Hb level is not immediate. The combination of daily ESA and IV iron administration has proven to be effective in increasing Hb levels and preventing transfusion in multiple clinical scenarios that often require blood transfusion including those with burn injury,[21] gastrointestinal hemorrhage,[2] thoracic, splenic and exsanguinating extremity trauma,[22,23] chemotherapy,[24] and total hip arthroplasty.[25]

Functional iron deficiency is common in critically ill and hospitalized patients related to "anemia of inflammation."[26] Inflammation induces up regulation of hepcidin, an acute-phase protein made by the liver, which reduces iron availability by decreased absorption of iron across the gastrointestinal tract, inhibition of iron efflux through ferroportin (the sole known iron exporter of enterocytes, macrophages, and hepatocytes) and decreased release of

iron from macrophages and the reticuloendothelial system.

There is no definitive diagnostic laboratory method by which iron deficiency can be established in critically ill JW patients since most diagnostic iron studies are altered related to the inflammatory response. Iron supplementation is administered in all JW patients in an effort to increase endogenous erythropoiesis and maximize efficacy of ESAs. IV iron administration is used, given that high hepcidin concentrations impair enteral iron absorption by the gut. Our institutional protocol uses IV iron sucrose 100 mg daily for 10 days or until the Hb concentration is stable at more than 7 g/dL. Higher daily doses may be considered in patients with severe life-threatening anemia.

Treatment of other anemia etiologies

Nutritional deficiencies are additional potential etiologies of anemia. If previous diagnostic assessment of other anemia etiologies has not been completed, then assessment for folate and vitamin B12 deficiency is recommended. Empiric folate and B12 supplementation is administered if diagnostic laboratory testing is not possible.

HBOCs

HBOC (Table 1) administration is considered in JW patients with severe life-threatening anemia. Recently, Hemopure (HBOC-201, purified, cross-linked acellular bovine Hb in a modified lactated Ringer's solution, OPK Biotech, Cambridge, MA) was the only HBOC available for clinical use requiring patient consent, institutional review board approval, and Food and Drug Administration (FDA) emergency investigational new drug (IND) approval for use in individual patients. At present, it is uncertain whether Hemopure will continue to be made available for clinical use in patients in the United States.

Hemopure is approved for human use in South Africa for the treatment of adult surgical patients who are acutely anemic and is also approved for use as an oxygen carrier in Russia. Englewood Hospital has initiated an "Expanded Access Study of HBOC-201 (Hemopure) for the Treatment of Life-Threatening Anemia" to provide Hemopure to patients with life-threatening anemia (Hb < 6 g/dL) when allogeneic transfusion is not an option (NCT01881503).[27]

Sanguinate (Prolong Pharmaceuticals, South Plainfield, NJ) is a bovine PEGylated carboxyHb and was developed to combine the beneficial functions of a carbon monoxide-releasing molecule (to promote anti-inflammatory and antivasoconstriction effects) with an oxygen transfer agent. A Phase I safety study was recently completed, and no serious adverse events were reported.[28] A Phase Ib trial in sickle cell patients is currently under way (NCT01374165). Sanguinate is also available for use under an expanded access emergency IND program for treatment of JW patients with severe anemia.

Our experience with HBOCs for JW patients is with Hemopure. Hemopure can bridge between life-threatening anemia and the time it takes for ESA and/or iron therapy to restore endogenous Hb levels. Hemopure does not require ABO screening; is stable at room temperature for 3 years; and does not require refrigeration, warming, or reconstitution. One unit of Hemopure includes 32.5 g of polymerized bovine Hb in approximately 225 to 250 mL of a solution similar to lactated Ringer's, increases the plasma Hb level 0.63 g/dL,[29] and has a half-life of approximately 19 hours. Therefore, Hemopure provides a transient improvement in oxygen delivery. Hemopure administration is not FDA-approved.

Concerns with HBOC administration include the vasoactive side effects associated with nitric oxide scavenging that may lead to hypertension, coronary artery vasoconstriction, MI, and increased mortality. This higher death rate is potentially of great concern. A meta-analysis reviewed data on death and MI as outcome variables in 16 trials in adult patients (n = 3711) involving five different

TABLE 1. HBOCs status as of 2014

Product class	Product	Company	Technology	Status
Cross-linked Hb	HemAssist ($\alpha\alpha$Hb, DCLHb)	Baxter US Army	Cross-linked Hb Cross-linked Hb	Discontinued; safety; increased mortality
	rHb 1.1	Somatogen	Recombinant Hb	Discontinued; safety; hypertension
	rHb 2.0	Baxter	Recombinant Hb	Discontinued; safety
Polymerized Hb	PolyHeme	Northfield Laboratories	Gluteraldehyde, pyridoxal human Hb	Discontinued; safety
	HBOC-201 (Hemopure)	OPK Biotech	Gluteraldehyde bovine Hb	Discontinued, approved for use in South Africa and Russia
	HemoLink	Hemosol	Polymerized human Hb	Discontinued; safety; MI
Conjugated Hb	PHP	Apex Bioscience	PEG-human Hb	Discontinued
	PEG-Hb	Enzon	PEG-bovine Hb	Discontinued
	Hemospan/MP4	Sangart	PEG-human Hb	Discontinued; no efficacy
	Sanguinate	Prolong Pharmaceuticals	PEGylated carboxyhemoglobin bovine	Phase I clinical trials

PEG = polyethylene glycol; PHP = pyridoxylated Hb polyoxyethylene conjugate.

HBOCs in varied patient populations.[30] They reported a significant increase in the risk of death (164 deaths vs. 123 deaths; relative risk, 1.30; 95% CI, 1.05-1.61) and MI (59 MIs vs. 16 MIs; relative risk, 2.71; 95% CI, 1.67-4.40).

There are, however, some limitations to this meta-analysis[31]: multiple products (HemAssist, PolyHeme, Hemolink, Hemopure, Hemospan; Table 1) were included in this analysis, lack of consistent monitoring of cardiac events in these studies, lack of consistent treatment in the perioperative period to prevent cardiac events in the surgical studies, no identification of specific cardiac risk in patients enrolled in these studies, and lack of control for risk of myocardial events and mortality that may have been related to allogeneic RBC transfusion.[32-34] Hemopure may also lead to methemoglobinemia, which is a minimal risk with 1- to 2-unit transfusions and is rarely symptomatic unless methemoglobin is more than 10%.

Given the lack of alternatives, time needed for ESAs and iron to augment erythropoiesis, and the high mortality of severe anemia in JW patients, Hemopure may be administered under compassionate-use FDA guidelines.[35] The FDA requires that we provide a standardized slideset (provided by the FDA when emergency IND approved) to review all possible associated risks and complications of HBOCs (including increased mortality and increased MI rates in prior human clinical trials) with the JW patient when obtaining informed consent for HBOC administration.

Under the FDA's Expanded Access program, Hemopure has been used to treat severe anemia in JW patients after induction chemotherapy for leukemia,[36] burns,[37] trauma,[38] and autoimmune hemolytic anemia.[39] Hemopure or HBOCs are not a replacement for human allogeneic blood transfusion, but in cases of life-threatening anemia in JW patients for which blood transfusion is not an option, it may be life-saving.[35,40]

Minimizing phlebotomy and diagnostic blood testing

Prevention of blood loss related to phlebotomy for diagnostic laboratory testing is critically important in patients with severe anemia. In our protocol, phlebotomy is limited to a maximum of 0.3 to 1 mL daily (using arterial or venous blood gas syringe) with preference for every other day laboratory testing if possible. Blood gas testing provides adequate diagnostic testing to include Hb and methemoglobin percentage. For other diagnostic testing, pediatric tubes should be used.

SEVERE ANEMIA PROTOCOL FOR JW PATIENTS

We established a clinical protocol for the treatment of severe anemia in JW patients to provide early standardized treatment (Fig. 2).

ESAs and iron treatment

For any JW patient with Hb level of less than 7 g/dL, ESA (epoetin alfa, Procrit, Amgen, Inc., Thousand Oaks, CA) is administered daily (40,000 units) until the Hb level is more than 7 g/dL. ESA dosing is reduced thereafter and long-term ESA needs are determined on an individual basis. Iron sucrose 100 mg IV is administered concurrent with ESA therapy daily until the Hb level is more than 7 g/dL. Subsequently, oral iron may be substituted if gastrointestinal function is adequate. Venous duplex monitoring is considered in patients with high risk for venous thromboembolism, as ESA use is associated with increased venous thromboembolism rates[41] in cancer[42] and critical illness.[14]

HBOC indications and administration

HBOC administration is considered in JW patients when the Hb level is less than 5 or 5 to 7 g/dL with associated symptoms of hypoperfusion. One unit of Hemopure should increase the Hb level by 0.63 mg/dL, but the half-life of 19 hours is short and must be considered in the daily assessment of the severity of anemia.

Hemopure can be administered via standard IV tubing and should be infused slowly over 4 hours (60 mL/hr) to avoid potential vasoconstrictive effects. If hypertension develops, the infusion should be held until resolution of the hypertension. Methemoglobin must be monitored, and any methemoglobin more than 10% and/or development of cyanosis or shortness of breath should caution additional transfusions of Hemopure. Methylene blue can be considered to reverse methemoglobinemia associated with cyanosis, altered mental status, or respiratory depression.

HBOC institutional review board or FDA approval and informed consent

HBOC use for the treatment of severe life-threatening anemia in JW patients requires FDA approval by physician request for an individual-patient emergency IND, and the manufacturer must agree to provide the HBOC free of charge.[43] Once the decision has been made that HBOC transfusion is indicated, the following steps are required to ensure the safe and responsible acquisition of HBOC (typically, this process takes 24-48 hours, so anticipation of the need for HBOC is essential):

1. Contact a physician in your institution familiar with HBOC transfusion;
2. Contact manufacturer (OPK Biotech, Prolong Pharmaceuticals) to confirm that they are willing to supply HBOC free of charge;
3. Obtain FDA approval for administration via compassionate use protocol (emergency IND) http://www.fda.gov/Drugs/DevelopmentApprovalProcess/

HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/ucm107434.htm;

4. Obtain institutional review board approval;
5. Obtain informed consent from the patient or representative.

Case series of patients treated with anemia protocol for JW patients

The following cases highlight the use of HBOC as an oxygen carrier bridge until ESAs and iron supplementation help to augment endogenous erythropoiesis. Six JW patients were treated with this protocol including HBOC infusion over 2 years (2012-2014; Table 2). Twenty-eight units of Hemopure were transfused. Figure 3A shows the progressive, but not immediate, Hb increase with the use of Hemopure as a temporary bridge until the effects of ESA and/or iron were evident in nonsurgical patients. Figure 3B demonstrates time-dependent Hb changes in patients who require surgery. In these cases, total Hb was estimated with blood gas Hb measurements rather than separate cellular Hb (complete blood count) and free Hb (reflecting HBOC Hb) tests. In Patient 2, this protocol was used to achieve a Hb level of more than 7 g/dL before total colectomy for an ulcerative colitis flare with ongoing bleeding. In Patient 3, 2 units of Hemopure were administered in the operating room during an above-knee amputation. Hb increased to more than 7 g/dL typically 6 to 10 days after protocol initiation.

There were no direct complications associated with Hemopure administration. One of the 28 transfusions was associated with hypertension and resolved with slowing of the transfusion rate. The highest methemoglobin was 9.3%, and no related symptoms were evident. Five patients had duplex surveillance with only one positive for an infrapopliteal deep venous thrombosis that was not treated. Interestingly, two patients with ARMS scores of 6 to 7 (50% predicted mortality) survived, while the patient with the highest score (8; >83% predicted mortality) died.

TABLE 2. Clinical characteristics of JW patients treated with anemia protocol

Patient	Age (years)	Sex	Cause of anemia	Starting Hb (g/dL)	Units of Hemopure transfused	Highest methemoglobin level	Surgery	DVT scan performed	DVT present	Auckland anemia mortality risk score	Outcome
1	70	Female	Epistaxis, ESRD	4.2	4	9.3	None	No	NA	5	Home
2	58	Female	GI bleed, surgery, ESRD	4.2	9	4.4	Above-knee amputation	Yes	None	8	Death from sepsis
3	55	Female	Ulcerative colitis flare, azathioprine, hydroxyurea	4.1	4	Not recorded	Total colectomy	Yes	None	3	Home
4	85	Female	GI bleed on dabigatran	4.4	2	4.3	None	Yes	Posterior tibial	7	Home
5	42	Female	Chronic anemia with menses	3.5	3	4.9	None	Yes	None	2	Home
6	71	Female	Sepsis and surgery	4.0	3	5.4	Above knee amputation	Yes	None	6	Rehab

DVT = deep venous thrombosis; ESRD = end-stage renal disease; GI = gastrointestinal.

FUTURE THERAPIES FOR ANEMIA IN JW PATIENTS

Since a number of studies have confirmed that high hepcidin concentrations contribute to the anemia of inflammation, newer treatment strategies are now focusing on antihepcidin therapeutics.[44,45] Hepcidin levels increase to extremely high levels in critically ill trauma patients and are positively correlated with the duration of anemia.[46] Interestingly, it has been shown that a single dose of ESA can result in rapid suppression of blood hepcidin concentrations.[47]

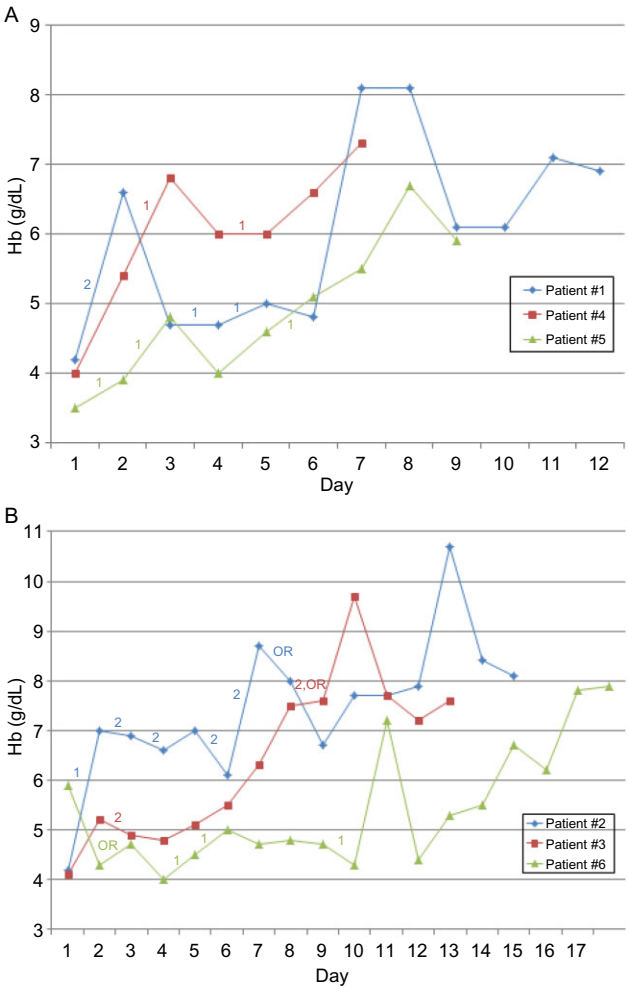

Fig. 3. (A) Hb levels in three JW patients from start of the severe anemia protocol until discharge. The timing and number of units of Hemopure are indicated by the corresponding number on that day. Patient numbers match those in Table 1. (B) Hb levels in three JW patients from start of the severe anemia protocol until discharge who underwent surgery during this time. Day on which surgery took place is indicated by the OR symbol. The timing and number of units of Hemopure are indicated by the corresponding number on that day. Patient numbers match those in Table 1.

Ongoing clinical trials are testing the efficacy of hepcidin antagonists. The hepcidin inhibitor NOX-H94 (a structured mirror-image RNA oligonucleotide, Spiegelmer) has completed Phase I trials in healthy humans and is currently in a Phase II trial to treat anemia associated with chronic disease.[48] LY2787106, a humanized antibody designed to bind and neutralize hepcidin, is being investigated in a Phase I clinical trial in patients with cancer-associated anemia (NCT01340976). Anticalin (therapeutic protein derived from human lipocalins) PRS-080 specifically binds human hepcidin with subnanomolar affinity, and clinical trials are scheduled.[49,50]

Is it time for antihepcidin treatment for JW patients with severe anemia? We will await the results of these clinical trials to determine the efficacy of hepcidin antagonists as novel therapeutics for iron-restricted anemias including anemia of inflammation (formerly known as "anemia of chronic disease") and continue our search for optimal therapies of anemia in JW patients.

In conclusion, JW patients with severe life-threatening anemia present a challenging clinical problem. Methods to augment endogenous erythropoiesis and minimize phlebotomy can successfully manage anemia. In situations of life-threatening critical anemia, HBOCs can bridge patients until endogenous Hb levels are adequate with support from pharmacologic treatment strategies (ESAs, iron, folate, vitamin B12). The use of a severe anemia protocol for JW patients can serve as a foundation for management of these challenging patients.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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