Low-dose erythropoietin treatment is not associated with clinical benefits in severely anaemic Jehovah's Witnesses: a plea for a change

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Background. Jehovah's Witnesses who refuse blood transfusion have high mortality. Erythropoietin (EPO) has been used as an alternative to blood transfusion. The optimal dosing of EPO in anaemic Jehovah's Witnesses is unknown. The aim of our study was to evaluate the clinical benefits of treatment with a low dose (<600 IU/kg/week) of epoietin beta (EPO-β).

Materials and methods. This was an observational study, retrospectively considering a 10-year period during which 3,529 adult Jehovah's Witnesses with a total of 10,786 hospital admissions were identified from databases of four major public hospitals in New Zealand. Patients with severe symptomatic anaemia (haemoglobin <80 g/L) who were unable to tolerate physical activity were included in the study. Patients treated without EPO were assigned to the conventional therapy group and those treated with EPO to the EPO treatment group.

Results. Ninety-one Jehovah's Witnesses met the eligibility criteria. Propensity score matching yielded a total of 57 patients. Patients treated with conventional therapy and those treated with EPO had similar durations of severe anaemia (average difference 6.25 days, 95% confidence interval [CI]: −3.77-16.27 days; p=0.221). The mortality rate among Jehovah's Witnesses treated with conventional therapy was 4.68 per year (95% CI: 2.23-9.82), while that in those treated with EPO was 2.77 per year (95% CI: 0.89-8.60). Treatment with EPO was associated with a mortality ratio of 0.59 (95% CI: 0.1-2.6; p=0.236). Both groups of patients had similar in-hospital survival (p=0.703).

Discussion. Treatment with low-dose EPO-β was not associated with either shorter duration of severe anaemia or a reduction in mortality.

Keywords: Jehovah's witnesses, severe anaemia, erythropoietin, morbidity, mortality.

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Introduction

Severely anaemic Jehovah's Witnesses who refuse blood transfusion on religious grounds have high mortality and morbidity[1-4]. Erythropoietin (EPO), an erythropoiesis-stimulating agent, can be used as an alternative to blood transfusion[5]. EPO-1 and EPO-2 studies showed that administration of epoetin alfa (EPO-α) at the dose of 40,000 units/week for 3 weeks in critically ill anaemic patients reduced exposure to allogeneic red blood cell transfusion and the amount of blood transfused[6,7]. EPO has other pleiotropic biological effects including anti-apoptotic, cytoprotective, angiogenic and anti-inflammatory actions mediated through EPO receptors located on neurones, cardiomyocytes, vascular endothelium and other tissues[8-10]. The EPO-3 randomised controlled trial demonstrated a survival advantage for patients treated with EPO compared with those given a placebo in critically ill trauma patients[11]. However, these large trials excluded Jehovah's Witnesses[12]. Biological responses to EPO and optimal dosing in severely anaemic Jehovah's Witnesses remain unknown[13]. Recently, Heh-Foster *et al*. conducted a retrospective, observational study and found no association between the rate of haemoglobin (Hb) recovery and any dose of EPO used in Jehovah's Witnesses[14]. However, the rate of Hb recovery might be influenced not only by the use of EPO but also by other important covariates that may differ between patients treated with EPO and those who are not. Propensity score matching must be included in the study design to adjust for pre-treatment characteristics in Jehovah's Witnesses. The aim of this study was to assess the clinical benefits of treatment with a low dose (<600 IU/kg/week) of epoietin beta (EPO-β) in propensity score matched, severely anaemic Jehovah's Witnesses.

Material and methods

This was a multicentre, retrospective, observational study. Adult (age ≥15 years) Jehovah's Witness patients were identified based on their self-reported religious status from databases of four major New Zealand public

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hospitals: Auckland City, North Shore, Middlemore and Waikato, over a period of 10 years (August 1998 to August 2008). The exclusion criteria were Hb concentration >8.0 g/dL during hospital admission, patients' palliative care status, end-stage kidney disease treated with continuous ambulatory peritoneal dialysis or haemodialysis, and treatment with a high dose of EPO-β (≥600 IU/kg/week). Patients younger than 65 years of age without cardiovascular and respiratory diseases who had mild symptoms of acute anaemia, including fatigue, weakness, shortness of breath and muscle cramps, who were able to tolerate physical activity were also excluded. The primary end-points of this study were duration of severe anaemia (Hb< 80 g/L) and in-hospital mortality. The secondary end-points were the weekly dose of EPO-β, nadir Hb level, in-hospital anaemia-related complications including death, operations for complications, duration of the index hospital admission, hospital readmission rate and the total duration of hospital stay. Patients' clinical data were obtained from the patients' clinical and operation notes, radiological image reports, laboratory tests results, and medication charts. Patients treated without EPO were assigned to the conventional therapy group whereas patients treated with low doses (<600 IU/kg/week) of EPO-β were assigned to the EPO group.

The study was approved by the New Zealand Ministry of Health Northern X Regional Ethics Committee (NTX/07/77/EXP). The approval included a waiver of informed consent.

Statistical analysis

The Stata Special Edition (version 13) software package (Statacorp LP, College Station, TX, USA) was used for the statistical analysis. Categorical variables are expressed as an absolute number (n) and percentage (%). Fisher's exact test was used for comparison of groups on categorical variables. Continuous variables are presented as the median and interquartile range. Two-sample Wilcoxon rank-sum (Mann-Whitney) test was used for comparison of continuous variables. p-values <0.05 were accepted as statistically significant. Propensity score matching with the nearest neighbour was performed to adjust for baseline pre-treatment differences[15]. All baseline pre-treatment characteristics, which included age, gender, ethnicity, diabetes mellitus, hypertension, dyslipidaemia, angina, previous myocardial infarction, heart failure, cardiac arrhythmia, past history of deep vein thrombosis or pulmonary embolism, pulmonary pathology, stroke, chronic kidney disease, depression, type of hospital admission (acute or elective), admission department, Hb on admission to hospital and nadir Hb concentration, were included in the propensity score model as covariates and duration of severe anaemia as the outcome variable. The average treatment effect on the treated (ATT) between the groups was estimated using probit regression. The standard error (SE) of the difference and 95% confidence interval (95% CI) of the difference was calculated by the bootstrap method. Propensity score matched groups were compared on primary and secondary points of outcome. Early mortality incidence rate (mortality), mortality difference and mortality ratio were calculated and compared using in-hospital mortality and the duration of hospital stay for patients treated with conventional therapy and those treated with EPO. Cox proportional hazards regression with adjustment for imbalance in propensity score matched groups was used to estimate in-hospital survival and morbidity of severely anaemic Jehovah's Witnesses.

Results

Study population

Over the 10-year observation period, 3,529 Jehovah's Witnesses had 10,786 hospital admissions at the four hospitals involved in the study. Of these patients, 3,438 were excluded from this study. Most of the patients were excluded because they had Hb concentrations above 8.0 g/dL during their hospital admissions (n=3,419). Other patients who were excluded from the study were cancer patients receiving palliative care (n=2), healthy patients younger than 65 years of age who had mild symptoms of anaemia and who were able to mobilise independently (n=5), patients with end-stage kidney disease treated with continuous ambulatory peritoneal dialysis or haemodialysis (n=10), and patients with acute haemorrhagic anaemia who were treated with high doses of EPO-β (n=2). A total of 91 Jehovah's Witnesses were, therefore, included in the study (Figure 1).

There were 64 Jehovah's Witnesses in the conventional treatment group and 27 in the EPO treatment group. The decision regarding whether a Jehovah's Witness was to be treated with EPO depended largely on the clinical situation and treatment care logistics. Jehovah's Witnesses with stable, severe life-threatening anaemia were treated with EPO. In contrast, Jehovah's Witnesses with non-life-threatening anaemia and those with ongoing bleeding were not considered as candidates for EPO therapy. Foreign Jehovah's Witnesses required written permission from their referring country's Ministry of Health or patients' visit sponsors before initiation of treatment with EPO.

Patients' demographics and pre-treatment characteristics are presented in Table I. Propensity score matching yielded 41 patients in the conventional therapy group and 16 patients in the EPO treatment group. The median dose of EPO-β was 235 units/kg/week (range: 94-484 units/kg/week). The EPO dosing regimen was selected by lead clinicians, often after

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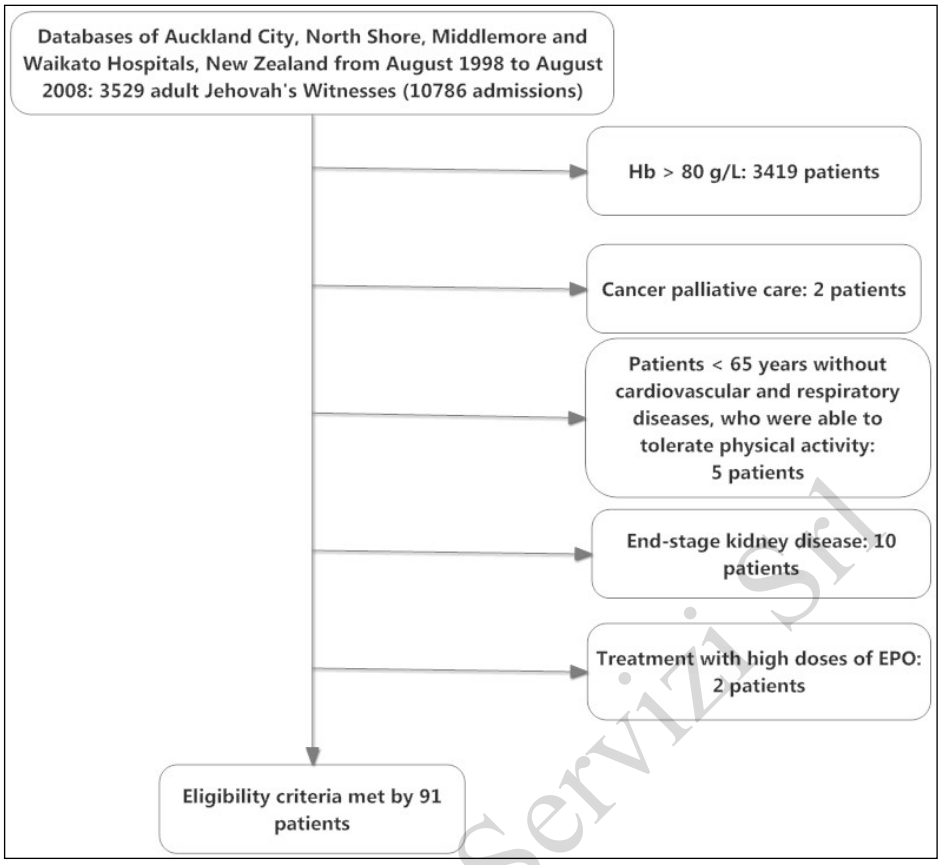

Figure 1 - Flowchart of the selection of severely anaemic Jehovah's Witness patients in the study.
Hb: haemoglobin; EPO: erythropoietin.

consultation with hospital pharmacists. In five (31%) patients, haematologists administered EPO treatment and monitored the bone marrow response to EPO by calculating the erythrocyte production index.

The average duration of severe anaemia in the unmatched conventional therapy group was 10.56 days and in the EPO treatment group, 4.39 days, a difference of 6.17 days (SE: 1.6 days; T-statistics: 3.86). Following propensity score matching, the ATT of duration of severe anaemia in the EPO treated patients decreased to 4.31 days (between groups difference: 6.25 days; SE: 2.15; T-statistics: 2.90). Bootstrap ATT of duration of severe anaemia difference was 6.25 days (SE: 5.11 days; 95% CI: –3.77-16.27 days; p=0.221).

Despite propensity score matching, the conventional therapy group and patients treated with low-dose EPO remained unbalanced with regards to nadir Hb level (Table II). The aetiology of anaemia was similar in both groups of matched patients (Table III), as were stimulation of haematopoiesis and systemic haemostatic medications used (Table IV). Apart from treatment with EPO, both groups of patients without ongoing bleeding received the same general supportive care including intravenous fluid resuscitation and maintenance, and nutritional support[16]. Infective complications in both groups were treated with intravenous or oral antibiotics and radiological interventional or surgical drainage procedures if appropriate.

There were seven deaths (17%) among patients in the conventional therapy group and three (19%) among the EPO-treated patients (odds ratio [OR]: 0.4; 95% CI: 0.1-2.9; p=0.368] (Table V). The EPO-treated patients had a higher proportion of infective complications (OR: 6.0; 95% CI: 1.1-34; p=0.041), including pneumonia (OR: 7.6; 95% CI: 1.5-39.8; p=0.016), than the conventional therapy group. Compared with the conventional therapy group, the EPO-treated patients also had longer index hospital admissions, with a median duration of stay of 25.5 days (compared with 8 days for the conventional therapy group) (p=0.006), and longer total duration of stay in hospital (29.5 days compared with 10 days) (p=0.008). All other complications were equally distributed between the conventional therapy and EPO treatment groups (Table V).

The mortality rate of severely anaemic Jehovah's Witnesses treated with conventional therapy was 4.68

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Table I - Demographic and pre-treatment characteristics of Jehovah's Witnesses treated with conventional therapy or with EPO.

Characteristics	Conventional therapy N=64, n (%)	EPO treatment N=27, n (%)	p-value
Age (median, interquartile range, years)	65 (44-74.5)	63 (51-71)	0.677
Gender, n (%)			
Male	24 (38)	13 (48)	
Female	40 (62)	14 (52)	0.361
Ethnicity, n (%)			
European	42 (66)	18 (67)	1
Maori	13 (20)	4 (15)	0.769
Others	9 (14)	5 (19)	0.751
Comorbidities, n (%)			
Diabetes mellitus	13 (20)	8 (30)	0.415
Hypertension	33 (52)	14 (52)	1
Dyslipidaemia	23 (55)	9 (53)	1
Angina	16 (25)	7 (26)	1
Previous myocardial infarction	14 (22)	2 (7)	0.135
Heart failure	8 (13)	7 (26)	0.131
Cardiac arrhythmia	12 (19)	5 (19)	1
Stroke/TIA	9 (14)	4 (15)	1
DVT/PE	1 (2)	1 (4)	0.508
Pulmonary pathology[¶]	20 (31)	5 (19)	0.305
Chronic renal failure[Ψ]	12 (19)	8 (30)	0.276
Depression	6 (9)	5 (19)	0.292
Admitting services, n (%)			
General medicine	15 (23)	9 (33)	
Cardiovascular surgery[§]	8 (13)	2 (7)	
General surgery and urology[χ]	13 (20)	7 (26)	
Orthopaedic surgery	17 (27)	9 (33)	
Obstetrics and gynaecology[ϕ]	11 (17)	0 (0)	0.132
Hospital admission type, n (%)			
Acute	48 (75)	21 (78)	
Elective	16 (25)	6 (22)	1
Hb on admission (median, interquartile range, g/dL)	113 (84-126)	111 (80-126)	0.99
Nadir Hb, (median, interquartile range, g/dL)	71 (61-77)	55 (46-62)	<0.01*

[¶] Includes asthma, chronic obstructive pulmonary disease, bronchiectasis, pulmonary tuberculosis; [Ψ] includes mild and moderate chronic renal failure; [§] includes coronary artery bypass surgery, heart valves repair and replacement, lower limb amputation, femoral endartectomy, lower extremity embolectomy; [χ] includes Roux-en-Y gastrojejunostomy, pancreatic necrosectomy, abdominal adhesiolysis, ventral incisional hernia repair, colectomy, mastectomy and dissection of axillary nodes, debridement of leg ulcers and skin graft, incision and drainage of subcutaneous abscess, haemorrhoid banding, transurethral urinary bladder tumour resection, stitching scalp laceration; [Ψ] includes spinal decompression and fusion, dynamic hip screw, hemiarthroplasty for neck of femur fracture, total hip arthroplasty, open reduction and internal fixation of femur fracture, total knee arthroplasty; [ϕ] includes dilatation and curettage of uterine cavity for retained products of conception, manual removal of placenta for post-partum haemorrhage, control of haemorrhage and vaginal laceration repair, transabdominal hysterectomy and bilateral oophorectomy, open salpingectomy for ectopic pregnancy. * $p<0.05$. TIA: transient ischaemic attack; DVT: deep vein thrombosis; PE: pulmonary embolism; Hb: haemoglobin.

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Table II - Baseline characteristics of Jehovah's Witnesses treated with the conventional therapy and with EPO after propensity score matching.

Characteristics	Conventional therapy N=41, n (%)	EPO treatment N=16, n (%)	p-value
Age (median, interquartile range, years)	65 (29-84)	63 (51-70)	0.563
Gender, n (%)			
Male	14 (34)	7 (44)	
Female	27 (66)	9 (56)	0.551
Ethnicity, n (%)			
European	29 (71)	8 (50)	1
Maori	9 (22)	3 (19)	0.769
Others	3 (7)	5 (31)	0.074
Comorbidities, n (%)			
Diabetes mellitus	12 (29)	6 (38)	0.545
Hypertension	23 (56)	11 (69)	0.549
Dyslipidaemia	23 (56)	9 (56)	1
Angina	13 (32)	5 (31)	1
Previous myocardial infarction	12 (29)	1 (6)	0.084
Heart failure	5 (12)	4 (25)	0.25
Cardiac arrhythmia	9 (22)	4 (25)	1
Stroke/TIA	7 (17)	2 (13)	1
DVT/PE	0 (0)	0 (0)	--
Pulmonary pathology[¶]	12 (29)	3 (19)	0.517
Chronic renal failure[Ψ]	7 (17)	6 (38)	0.158
Depression	2 (5)	2 (13)	0.312
Admitting services, n (%)			
General medicine	9 (22)	7 (44)	
Cardiovascular surgery[§]	8 (20)	2 (13)	
General surgery and urology[χ]	8 (20)	3 (19)	
Orthopaedic surgery	9 (22)	4 (25)	
Obstetrics and gynaecology[ϕ]	7 (17)	0 (0)	0.302
Hospital admission type, n (%)			
Acute	32 (78)	14 (88)	
Elective	9 (22)	2 (13)	0.71
Hb on admission (median, interquartile range, g/dL)	115 (71-139)	107 (80-126)	0.929
Nadir Hb, (median, interquartile range, g/dL)	72 (53-79)	55 (40-62)	<0.01*

[¶] Includes asthma, chronic obstructive pulmonary disease, bronchiectasis, pulmonary tuberculosis; [¥] includes mild and moderate chronic renal failure; [§] includes coronary artery bypass surgery, heart valve repair and replacement, lower limb amputation, femoral endartectomy, lower extremity embolectomy; [χ] includes Roux-en-Y gastrojejunostomy, pancreatic necrosectomy, abdominal adhesiolysis, ventral incisional hernia repair, colectomy, mastectomy and dissection of axillary nodes, debridement of leg ulcers and skin graft, incision and drainage of subcutaneous abscess, haemorrhoid banding, transurethral urinary bladder tumour resection, stitching scalp laceration; [Ψ] includes spinal decompression and fusion, dynamic hip screw, hemiarthroplasty for neck of femur fracture, total hip arthroplasty, open reduction and internal fixation of femur fracture, total knee arthroplasty; [ϕ] includes dilatation and curettage of uterine cavity for retained products of conception, manual removal of placenta for post-partum haemorrhage, control of haemorrhage and vaginal laceration repair, transabdominal hysterectomy and bilateral oophorectomy, open salpingectomy for ectopic pregnancy. * $p<0.05$.
TIA, transient ischaemic attack; DVT, deep vein thrombosis; PE, pulmonary embolism; Hb, haemoglobin.

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Table III - Aetiological types of anaemia in the conventional therapy group and EPO-treated patients.

	Conventional therapy N=41, n (%)	EPO treatment N=16, n (%)	p-value
Impaired production (iron-deficiency) anaemia	4 (10)	1 (6)	
Increased destruction (haemolytic) anaemia	1 (2)	2 (13)	
Acute haemorrhagic anaemia	10 (24)	5 (31)	
Acute haemorrhagic anaemia, post-operative	26 (63)	8 (50)	0.431

EPO: erythropoietin.

Table IV - Erythropoiesis stimulation and systemic haemostatic treatment administered to patients.

Treatment	Conventional therapy N=41, n (%)	EPO treatment N=16, n (%)	p-value
Iron (oral, intravenous)	28 (68)	13 (87)	0.306
Folic acid	3 (7)	4 (27)	0.074
Cyanocobalamin	3 (5)	1 (7)	1
FFP	0 (0)	1 (6)	0.281
Factor VIIa	0 (0)	1 (6)	0.281

EPO: erythropoietin; FFP: fresh-frozen plasma.

Table V - Complications in propensity score-matched Jehovah's Witnesses.

Complications	Conventional therapy N=41, n (%)	EPO treatment N=16, n (%)	OR	95% CI	p-value
Mortality	7 (17)	3 (19)	0.4	0.1-2.9	0.368
Haemorrhage	14 (34)	4 (25)	0.3	0.05-1.5	0.14
Hypovolaemic shock	7 (17)	7 (44)	2	0.4-9.8	0.372
Infective complications[¥]	20 (49)	13 (81)	6	1.1-34.0	0.041
Pneumonia	8 (20)	9 (56)	7.6	1.5-39.8	0.016
Wound infection	6 (15)	4 (25)	4.3	0.6-32.3	0.15
Cardiac arrhythmia	7 (17)	1 (6)	0.3	0.02-3.4	0.304
Angina	6 (15)	3 (19)	0.7	0.1-5.0	0.733
Ischaemic myocardial injury	8 (20)	2 (13)	0.5	0.06-3.6	0.474
Heart failure	9 (22)	7 (44)	1.7	0.4-7.7	0.503
Venous thrombotic complications[ϕ]	0 (0)	1 (6)			0.281
Neurological complications	4 (10)	2 (13)	0.6	0.1-6.0	0.653
Acute/acute-on-chronic renal failure	14 (34)	8 (50)	1.5	0.4-6.3	0.591
Delirium	8 (20)	2 (13)	0.3	0.04-2.8	0.321
Duration of index hospital admission (median, interquartile range, days)	8 (1-31)	25.5 (9-37)			0.006
Hospital readmission	6 (15)	4 (25)			0.443
Total duration of stay (median, interquartile range, days)	10 (2-37)	29.5 (10-42)			0.008

[¥] Includes pneumonia, wound infection, urinary tract infection, central venous line-related sepsis, intravenous line-associated cellulitis; [ϕ] includes deep vein thrombosis, portal vein thrombosis and pulmonary embolism.
EPO: erythropoietin; OR: odds ratio; CI: confidence interval.

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per year (95% CI: 2.23-9.82), whereas that of the corresponding patients treated with EPO was 2.77 per year (95% CI: 0.89-8.60). Treatment with EPO was associated with a difference in mortality of –1.91 (95% CI: –6.59-2.77) and a mortality ratio of 0.59 (95% CI: 0.1-2.6; p=0.236).

Cox proportional hazards regression adjusted for nadir Hb concentration showed that compared to conventional therapy, treatment with EPO was associated with a mortality hazards ratio (HR) of 0.19 (95% CI: 0.03-1.47; p=0.112). The test of proportional hazards assumption demonstrated that the HR was constant over the time of observation (χ^2=0.43, degree of freedom: 2, p=0.809). The two groups of patients (conventional therapy group and EPO-treated group) had similar in-hospital survival (p=0.703).

Discussion

We have shown that, in propensity score matched severely anaemic Jehovah's Witnesses, treatment with a low-dose of EPO-β compared with conventional therapy was not associated with a shortened duration of severe anaemia. The EPO-treated patients had a higher rate of infective complications, which could have been due to a confounding effect of the severity of anaemia**.** The two groups had similar morbidity and in-hospital survival.

EPO is a 165 amino-acid glycoprotein that is produced by renal capillary endothelial cells in response to hypoxia[8,17,18]. EPO binds to EPO receptors (EPOR) expressed in different cells including erythroid and non-erythroid cells. On early haematopoietic progenitors, the burst-forming unit erythroid and colony-forming unit erythroid, and also on proerythroblasts and basophilic erythroblasts, EPO binds to EPOR-EPOR homodimers and initiates JAK2/STAT5, phosphatidylinositol 3-kinase, RAS/MAP kinase and protein kinase C signalling pathways that endorse erythroid differentiation, survival and proliferation[8,19,20].

EPO has been used for decades to treat patients with anaemia caused by end-stage kidney disease and, more recently, to treat chemotherapy-induced anaemia, some haematological disorders and acute haemorrhagic anaemia[18,21,22].

Effects of EPO-based therapy on patients' blood transfusion requirements have been investigated in different clinical settings with contrasting results. In one study of critically ill patients, compared with placebo, subcutaneous administration of EPO-α at the dose of 40,000 units/week for 3 consecutive weeks did not reduce patients' exposure to allogeneic blood transfusion (ABT) (48.3% *vs* 46.0%, relative risk of 0.95, 95% CI: 0.85-1.06, p=0.34) and the amount of allogeneic red blood cell units transfused (4.3±4.8 units *vs* 4.5±4.6 units, p=0.42)[11]. However, a 2007 meta-analysis showed that, in critically ill anaemic patients, administration of any dose of EPO-α significantly reduced the odds of ABT (OR: 0.73, 95% CI: 0.64-0.84, I^2=54.7%), and the volume of ABT per patient (weighted mean difference –0.41 units per patient, 95% CI: –0.74 to –0.10, I^2=79.2%)[23].

Takami and Masumoto found that in anaemic patients undergoing cardiac surgery, subcutaneous administration of EPO at a dose of 24,000 IU/week before pre-operative autologous blood donations did not reduce patients' exposure to ABT[24]. Using blood transfusion triggers of Hb<60 g/L during cardiopulmonary bypass and Hb<85 g/L post-operatively, the authors showed that EPO was used in 55% of patients who avoided ABT, and in 50% of patients who underwent ABT (p=0.96)[24].

In colorectal cancer, surgical patients treated with the standard of care and patients who were treated with any dose of EPO, had similar requirements for ABT. In addition, the numbers of units of allogeneic red blood cells transfused per patient were equal in both groups[25].

In patients undergoing hip and knee surgery, those treated with any dose of EPO in combination with iron supplementation had a statistically higher post-operative Hb concentration and a reduced requirement for ABT when compared with the control group of patients treated with either cell salvage, pre-operative autologous blood donation, and/or iron therapy[26].

The erythropoiesis-stimulating effect of EPO in anaemic patients who declined ABT was studied recently in a retrospective cohort study[14]. Heh-Foster *et al.* found that, compared with patients not treated with EPO, EPO-treated patients had similar rates of Hb recovery regardless of the dose and duration of EPO treatment[14]. Our study agrees with these findings in that we found that a low-dose regimen of EPO had no clinical benefit in propensity score matched Jehovah's Witnesses who were severely anaemic. However, because of a lack of studies evaluating the effects of high-dose EPO treatment on patients' outcomes, clinical acumen suggests that severely anaemic Jehovah's Witnesses should continue to be treated with high-dose EPO until more evidence emerges, rather than not being treated.

Treatment with EPO may come with increased risks of infection. EPO-induced intracellular signalling in non-erythroid cells, including cells of the immune system, platelets and endothelial cells is mediated via a heterodimeric receptor composed of an EPOR subunit and a β common receptor subunit (aka CD131)[9,10,27]. In contrast to EPOR monodimers on erythropoietic cells, activation of EPOR-CD131 receptors located on the surface of cells other than erythroid progenitors requires high local concentration of EPO[27]. Binding of EPO to EPOR-CD131 receptors located on neutrophils weakens the production of reactive oxygen species

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which impairs intracellular killing of micro-organisms and attenuates inflammation[27]. The EPO-EPOR-CD131 interaction also inhibits transcription of nuclear factor-κB-dependent pro-inflammatory genes, including inducible nitric oxide synthase and tumour necrosis factor-α, which leads to reduced synthesis of these anti-microbial particles in macrophages[28]. Additionally, EPO impairs the functional activity of T helper-1 and T helper-17 cells. In experiments with systemic *Salmonella typhimurium* infection in mice, treatment with EPO impaired pathogen clearance and shortened survival[28]. Blocking EPO with a neutralising antibody lowered bacterial load in the spleen, indicating improved elimination of pathogens[29]. However, in the EPO-2 and EPO-3 trials investigating clinical benefits of recombinant human EPO (rhEPO) in critically ill trauma patients, the placebo groups and EPO-treated patients had similar mean Hb concentrations, above 90 g/L, and rates of infective complications[12]. In our study, the EPO-treated patients had a lower median nadir Hb concentration than the patients in the conventional therapy group (55 g/L, interquartile range: 40-62 g/L *vs* 72 g/L, interquartile range: 53-79 g/L; p<0.01) and a higher rate of infective complications than the conventional therapy group (OR: 6; 95% CI: 1.1-34; p=0.041). The higher rate of infective complications is most likely attributable to a confounding effect of severe anaemia rather than treatment with EPO, based on previous studies involving higher doses of EPO which did not show any increased susceptibility to infection[12]. Furthermore, we previously showed that Jehovah's Witnesses with severe anaemia who refused blood transfusion had higher infective complication rates than anaemic patients who underwent ABT[2].

In patients with end-stage kidney disease, treatment with EPO has been implicated in the development of thrombotic complications[30]. rhEPO is an athletic performance-enhancing drug that is banned in sport[31]. When athletes become dehydrated, rhEPO misuse may result in severe hyper-viscosity with a rise in haematocrit to as high as 80%, stroke, seizures, encephalopathy, pulmonary embolism, myocardial infarction, deep vein thrombosis and sudden death[31]. *Ex vivo* experiments have demonstrated that rhEPO treatment of blood obtained from young and healthy individuals results in increased platelet reactivity[32]. Aspirin can reverse EPO-induced platelet hyper-reactivity[33,34]. Patients in our study treated with EPO for severe life-threatening anaemia were not treated with aspirin or heparin. However, if patients' conditions stabilised and their Hb exceeded 70 g/L many were started on antiplatelet therapy if indicated and unfractionated heparin or low molecular weight heparin thromboprophylaxis. One patient in our study developed post-operative deep vein thrombosis, portal vein thrombosis and pulmonary embolism and was started on an infusion of unfractionated heparin. Subsequently this patient had excessive blood loss and developed severe anaemia. The heparin infusion was stopped and treatment with EPO was then initiated. No venous thrombotic complications were reported while patients received EPO treatment.

A cytoprotective effect of EPO has been consistently demonstrated in *in vitro* and animal models[35-37]. The paucity of observed biological effects of EPO in clinical studies can be explained by differences in physiology between experimental animals and humans, and different regimens of EPO therapy[38]. One of the few clinical trials in which a positive effect was observed was the EPO-3 study[11]. In critically ill trauma patients treated for 3 weeks with 40,000 units/week of EPO-α, the 29-day mortality was reduced by 49% (unadjusted HR: 0.51; 95% CI: 0.27-0.98; p=0.039), fully adjusted HR: 0.36 (95% CI: 0.18-0.74), best-fit adjusted HR: 0.38 (95% CI: 0.19-0.74). In the EPO-3 trial, the 42-day mortality unadjusted HR was 0.51 (95% CI: 0.27-0.95, p=0.030) and the adjusted HR was 0.35 (95% CI: 0.18-0.68), while the 140-day mortality unadjusted HR was 0.61 (95% CI: 0.37-1.03), and the adjusted HR was 0.41 (95% CI: 0.24-0.70)[12]. In our study, there was no evidence of an association between low-dose EPO treatment and mortality or morbidity, which included ischaemic myocardial injury, acute kidney injury and neurological complications. The negative findings in our study may be explained by the use of low doses of EPO (<600 IU/kg/week), as EPO doses ranging from 1,000 IU/kg to 5000 IU/kg/week have commonly been used in preclinical studies. Our study provides indirect support for the proposition that severely anaemic Jehovah's Witnesses should be treated with a high-dose regimen of EPO, iron, folate and cyanocobalamin[39].

One of the limitations of our study is due to the retrospective design. Because of the lack of previous studies evaluating the clinical benefits of EPO in severely anaemic Jehovah's Witnesses, it was not possible to determine whether hospital-based protocols existed to guide treatment of these patients. In addition, it is not clear how EPO-β doses were chosen and whether the dosing regimens took into account the presence of inflammation, platelet count or type of anaemia that patients had. Clinical studies evaluating high-dosage regimens of EPO treatment in severely anaemic Jehovah's Witnesses are urgently needed.

Conclusions

Treatment with low-dose EPO-β is not associated with a shortened duration of severe anaemia, decreased morbidity or improved survival of Jehovah's Witnesses. To potentially improve management and outcome of

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severely anaemic Jehovah's Witnesses, we therefore recommend that any EPO dosing regimens should include higher doses of EPO. Further studies of the efficacy of high-dose EPO in Jehovah's Witnesses with severe, life-threatening anaemia are warranted. In calculating EPO dosing requirements, the development of complications, such as sepsis, types of anaemia and platelet count should be taken into consideration, and vigilance maintained for such complications throughout treatment.

Authorship contributions

AMB contributed to the study conception, the acquisition, statistical analysis and interpretation of the data, and writing the manuscript. SA, PM, PN and WS contributed to data collection and interpretation, and writing the final version of the manuscript. CB contributed to data collection and interpretation and writing the manuscript.

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