Erythropoiesis is not equally suppressed in transfused males and females with β-thalassemia major: are there clinical implications?

Blood transfusion in β-thalassemia major aims to suppress ineffective erythropoiesis, limit morbidity from chronic anemia, and minimize bone changes from marrow expansion and extra-medullary hemopoiesis (EMH).[1] In the 1970s, blood transfusion was shown to suppress erythropoiesis.[2] It is uncertain how much blood should be transfused to each patient for optimal long-term health and to minimize complications. At present, all patients are transfused equally with no consideration given to gender.[3] There are limited data exploring differences between males and females. This study demonstrates important differences in pre-transfusion indices of erythropoiesis, descriptors of blood transfusion and the frequency of para-spinal EMH when patients are transfused according to current recommendations. These findings strongly support the notion that erythropoiesis is not being equally suppressed in males compared to females and hence that males are being under-transfused with current practices.

Extra-medullary hemopoiesis is the presence of hemopoietic tissue outside of the bone marrow, which occurs as a compensatory process in the setting of ineffective erythropoiesis.[1] Spinal cord compression is an uncommon but serious complication of para-spinal EMH. There is a clear male predominance (M:F ratio ≥4:1)[4,5] that has been documented without drawing attention to gender differences or the possible need for males to be treated more intensively to avoid this complication. We aimed to determine by using readily available laboratory tests whether there were differences in erythropoietic activity between male and female adult β-thalassemia major patients receiving a regular transfusion regimen in accordance with current international recommendations.[3]

All 126 adults (≥18 years) with β-thalassemia major at our center were eligible. All patients had been receiving an individualized and regular transfusion regimen and iron chelation therapy in accordance with current recommendations.[3] Patients' height and weight were recorded to estimate blood volume using Nadler's formula.[6] Blood samples were collected on the day of, and prior to, routine transfusion when it was expected that erythropoietic activity would be maximal. Blood samples were tested for hemoglobin (Hb), nucleated red blood cell (NRBC) and absolute and relative reticulocyte counts with a Beckman Coulter LH750 hematology analyzer. Blood films were examined for manual NRBC and reticulocyte counts with a light microscope. Serum ferritin and erythropoietin (EPO) levels were determined by immunoassay (Beckman Coulter DXi 800). We perform regular MRI at 1.5 Tesla (Siemens Avanto) to assess cardiac function and to monitor hepatic and cardiac iron loading using the T2* technique. Additional chest and abdomen imaging was assessed for the presence of para-spinal EMH. This study was approved by the Monash Health Ethics Committee. Informed consent was obtained from each patient to draw blood during routine clinical care. Data were analyzed using SAS software v.9.3 (SAS Institute, Cary, NC, USA). Comparisons between groups were performed using Mann-Whitney U-test for non-normally distributed continuous variables, Student t-test for normally distributed continuous variables, and χ^2 or Fisher's exact test as appropriate for categorical variables. Two-sided P=0.05 was considered statistically significant.

One hundred and sixteen patients (51 males and 65 females) provided pre-transfusion blood samples (Table 1). The 10 patients not included were either not available or declined testing. Males and females were of similar ages and were receiving blood over a similar inter-transfusion interval. Males were heavier, taller and had a higher esti-

Table 1. Summary and laboratory indices of pre-transfusion erythropoiesis for all patients.

Variable	Overall (n=116)	Male (n=51)	Female (n=65)	P
Age (years)	39.3±9.3	40.0±9.6	38.8±9.1	0.51
Weight (kg)	60.0±11.6	64.9±9.8	55.7±12.4	< 0.001
Height (cm)	159±10.5	166±8.3	153±8.1	< 0.001
Estimated annual transfused volume (L)	13.5 (10.1-13.5)	14.0 (14.0-14.0)	14.0 (10.0-14.0)	0.001*
Estimated annual transfused volume per kg (mL/kg)	215 (185-255)	202 (185-225)	225 (188-263)	0.028
Transfusion interval (weeks)	3.2±0.6	3.2±0.7	3.1±0.5	0.16
Number of RBC units per transfusion	3.0±0.63	3.2±0.76	2.9±0.48	0.006
Estimated patient blood volume** (L)	3.79±0.73	4.39±0.50	3.32±0.52	< 0.001
Hemoglobin (g/L)	98±8.9	98±9.9	99±8.1	0.36
Ferritin (mcg/L)	821 (604-1300)	754 (582-1139)	943 (639-1356)	0.11
Erythropoietin (mIU/mL)	58 (37-99)	72 (41-149)	52 (35-89)	0.006
Absolute automated reticulocyte count (10^9/L)	85 (33-146)	104 (63-171)	72 (33-127)	0.025
Absolute manual reticulocyte count (10^9/L)	39 (16-86)	39 (18-86)	39 (13-84)	0.77
Automated NRBC (number NRBC/100 WBC counted)	5 (0-34)	17 (0-54)	1 (0-20)	0.014
Manual NRBC (number NRBC/100 WBC counted)	6 (1-54)	21 (2-98)	3 (1-28)	0.003
Splenectomy	49.5%	61%	40%	0.031

*Data are mean ± standard deviation, median (interquartile range) or percentage. *Although the medians were the same for males and females, the interquartile ranges were different between groups. Transfused volumes are calculated on the assumption that each unit contains 260 mL red cells (Australia Red Cross Blood Service red cell unit mean volume = 259 ± 23 mL).[15] **Calculated using Nadler's formula for total blood volume (TBV): men TBV (mL) = 604 + [(367 x height^3(m^3) + (32.2 x weight (kg)]; women TBV = 183 + [(356 x height^3(m^3) + 33.1 x weight (kg)].[6]*

mated blood volume. Males were receiving more units of red blood cells per transfusion and had a higher annual transfusion volume of 14.0 liters (14.0-14.0) *versus* females 14.0 (10.0-14.0) (*P*=0.001); although the medians were the same, the interquartile ranges were different. However, when these values were corrected for weight, females were receiving a higher transfused volume per kg: 225 mL/kg (188-263) *versus* males 202 (185-225) (*P*=0.028). The incidence of splenectomy was higher in males (61% *vs.* 40% females; *P*=0.031). The mean pre-transfusion Hb was 98-99 g/L with no difference between males or females, thereby supporting our long-standing adherence to current recommendations (Table 1).[3] Erythroprotein (EPO) levels were higher in males: 72 mIU/mL (41-149) versus 52 (35-89); *P*=0.006. NRBCs were higher in males with both manual [21 (2-98) *vs.* 3 (1-28); *P*=0.003] and automated [17 (0-54) *vs.* 1 (0-20); *P*=0.014] counting. Males had higher absolute automated reticulocyte counts; however, there was no difference between automated and manual reticulocyte counts.

Analysis of differences between males and females based on splenectomy status (Table 2) demonstrated that estimated annual transfusion volumes were higher in males. EPO levels were significantly higher in splenectomized males compared to females: 77 mIU/mL (41-145) *versus* 48 (30-67); *P*=0.01. However, this difference was not observed in non-splenectomized patients. Ferritin levels were significantly higher in splenectomized females compared to males. There were no gender differences in Hb, reticulocyte or NRBC counts based on splenectomy status.

Six patients declined to have an MRI study. The incidence of para-spinal EMH was 13% (14 of 110). There were 11 cases in males and 3 cases in females (M:F ratio 3.66), resulting in an incidence in males and females of 22% (11 of 50) and 5% (3 of 60) (*P*=0.01), respectively. There had been no recent cases of epidural EMH with cord compression. Thirteen of the 14 patients with paraspinal EMH were splenectomized. An analysis excluding the 14 patients (11 males and 3 females) with EMH demonstrated that EPO levels remained significantly higher in males compared to females (*Online Supplementary Appendix*).

To our knowledge, this is the first report of a significant difference in EPO levels between males and females with β-thalassemia major. EPO levels were higher comparing all males to all females and in splenectomized males versus splenectomized females. Higher EPO levels were not seen in non-splenectomized males versus females; however, the number of males in this analysis may have been too small to detect any small difference (n=20). Previous studies reporting no difference between males and females had small numbers of patients and had assessed or included β-thalassemia intermedia patients.[7,8] Cazzola *et al.* reported that the mean pre-transfusion Hb was inversely related to EPO.[9] Despite different reference ranges for Hb, there is no difference in the EPO reference range for men and women, nor does it change with age.[10] The higher normal Hb levels in males compared to females results from the stimulation of erythropoiesis by androgens and its inhibition by oestrogens in females.[11] This suggests that males require a higher Hb level than females to achieve an equivalent suppression of erythropoiesis.

The number of NRBCs was higher in males with automated and manual counting, yet no difference was seen based on splenectomy status. Their presence in β-thalassemia major reflects increased erythropoietic activity due to ineffective erythropoiesis; however, the clinical significance of the absolute numbers of NRBCs is not well understood. Although we report a difference in automated reticulocyte counts between males and females, the manual reticulocyte counts were not significantly different nor

Table 2. Estimated annual transfused volumes and laboratory indices of pre-transfusion erythropoiesis for males and females based on splenectomy status.

Variable	Splenectomized patients			Non-splenectomized patients		
	Male (n=31)	Female (n=26)	*P*	Male (n=20)	Female (n=39)	*P*
Estimated annual transfused volume (L)	13.5 [8.1-18.0]	13.5 [6.7-13.5]*	0.008	13.5 (13.5-18.0)	13.5 (10.1-13.5)	0.015
Estimated annual transfused volume per kg (mL/kg)	193 (176-214)	195 (175-237)	0.69	215 (199-273)	237 (216-284)	0.15
Hemoglobin (g/L)	99±9.6	100±6.7	0.72	96±10.2	99±8.7	0.22
Ferritin (mcg/L)	658 (505-884)	1061 (719-1706)	0.01	793 (689-2233)	966 (621-1356)	0.43
EPO (mIU/mL)	77 (41-145)	48 (30-67)	0.01	72 (37-172)	56 (39-93)	0.28
Absolute automated reticulocyte count (10^9/L)	148 (97-220)	135 (92-167)	0.18	35 (13-94)	35 (8-74)	0.70
Absolute manual reticulocyte count (10^9/L)	43 (20-86)	40 (16-73)	0.88	25 (0.4-82)	20 (3.6-88)	0.84
Manual NRBC (number NRBC/100 WBC counted)	62 (20-178)	46 (15-95)	0.20	1 (1-5)	1 (0-1)	0.07
Automated NRBC (number NRBC/100 WBC counted)	36 (17-111)	34 (16-77)	0.56	0 (0-2)	0 (0-0)	0.43

*Data are mean ± standard deviation or median (interquartile range). *Data are median [range] as the interquartile ranges were identical at 10.1–13.5.*

were there differences based on splenectomy status. RBC interference (e.g. target cells, Howell Jolly bodies, anisocytosis) in β-thalassemia major samples makes it difficult to determine if there is any difference between the genders.[12] RBC interference and inter-observer variability contribute to the imprecision seen with manual reticulocyte counting.[13]

Despite the fact that males received a higher absolute annual blood volume, they received a lower estimated annual transfusion volume (mL/kg). In addition, the incidence of splenectomy was higher in males, consistent with the notion that males have less effective suppression of erythropoiesis from blood transfusion with a consequent higher rate of ongoing splenomegaly compared to females. This is consistent with our previous data demonstrating that males had significantly increased pre-transfusion erythropoiesis and experienced a smaller post-transfusion reduction in erythropoiesis compared to females.[14] Although this may be biological, a potential limitation of this study is the different proportion of splenectomized males compared to females.

The predominance of males with para-spinal EMH (M:F ratio 3.66) is consistent with previous observations and has important clinical implications. Issargisil *et al.*[4] reviewed 12 cases and reported a male:female ratio of 5:1. Salehi *et al.*[5] reviewed 42 cases and reported a male:female ratio of approximately 4:1 (phenotype not specified). The natural history of EMH is poorly understood. As males and females are currently transfused equally, it is likely that the male predominance relates to the unequal suppression of erythropoiesis. Adequate transfusion to prevent para-spinal EMH is important as treatment options are not always effective if spinal cord compression has occurred. That all but one of our 14 patients with EMH had been splenectomized suggests that EMH and splenectomy status were independent markers of the adequacy of blood transfusion.

Our data strongly support the notion that erythropoiesis is not equally suppressed in males and females, and that males are more prone to EMH with current transfusion practices. EPO may have a role in clinical practice for monitoring the suppression of erythropoiesis with transfusion. The optimal pre-transfusion Hb required to adequately suppress erythropoiesis and prevent the development of EMH may be higher for males than females. Further work is needed to clarify and confirm these findings and to evaluate the need for consideration of gender when developing transfusion and monitoring protocols for patients with β-thalassemia major.

Greg Hapgood,[1] Timothy Walsh,[2] Ronit Cukierman,[2] Eldho Paul,[3] Ken Cheng,[4] and Donald K. Bowden[2]

[1]Haematology Department, Monash Health; [2]Medical Therapy Unit (Thalassemia Service), Monash Health; [3]Monash Centre for Health Research and Implementation, School of Public Health and Preventive Medicine, Monash University; and [4]Radiology Department, Monash Health, Melbourne, Australia

Acknowledgments: the authors are grateful for the kind assistance from the nursing staff of the Medical Therapy Unit (Thalassemia Service) at Monash Health, in particular the nurse unit manager, Ms. Joanne Shaw. We are grateful for the assistance of the medical scientists in the haematology laboratory at Monash Health. In particular, Mr. Steven Jaskolowski provided important scientific input. We would like to thank the patients who willingly participated in this study.

Correspondence: greghapgood@hotmail.com
doi:10.3324/haematol.2014.118216

Key words: β-thalassemia mahor, erythropoiesis, males, females, transfusion.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Rachmilewitz E, Giardina P. How I treat thalassemia. Blood. 2011; 118(13):3479-3488.
2. Cavill I, Ricketts C, Jacobs A, et al. Erythropoiesis and the effect of transfusion in homozygous beta thalassemia. N Engl J Med. 1978; 298(14):776-778.
3. Cappellini M, Cohen A, Eleftheriou A, et al. Guidelines for the clinical management of Thalassemia. Second Edition, Nicosia, Cyprus; Thalassemia International Federation. Available from: http://www.thalassaemia.org.cy/educational-programme/publications.shtml.
4. Issargisil S, Piankjiagum A, Prawase W. Spinal cord compression in thalassemia. Report of 12 cases and recommendations for treatment. Arch Intern Med. 1981;141(8):1033-1036.
5. Salehi S, Koski T, Ondra S. Spinal cord compression in beta-thalassemia: case report and review of the literature. Spinal Cord. 2004; 42(2):117-123.
6. Nadler SB, Hidalgo JU, Bloch T. Prediction of blood volume in normal human adults. Surgery. 1962;51:224-232.
7. Dore F, Bonfigli S, Gaviano E, et al. Serum erythropoietin levels in thalassemia intermedia. Ann Hematol. 1993;67(4):183-186.
8. Manor D, Fibach E, Goldfarb A, et al. Erythropoietin activity in the serum of beta thalassemic patients. Scand J Haematol. 1986; 37(3):221-228.
9. Cazzola M, De Stefano P, Ponchio L, et al. Relationship between transfusion regimen and suppression of erythropoiesis in beta-thalassaemia major. Br J Haematol. 1995;89(3):473-478.
10. Miller M, Chandra M, Garcia J. Clinical Applications of Measurement of Serum Immunoreactive Levels of Erythropoietin. Ann N Y Acad Sci. 1985;459:375-381.
11. Jelkmann W. Regulation of erythropoietin production. J Physiol. 2011;589(6):1251-1258.
12. Paterakis G. Reticulocyte counting in thalassaemia. Clin Lab Haematol. 1996;18(1):17-28.
13. Beckman Coulter. The LH Reticulocyte Count and associated parameters. Bulletin 9231. Available from: https://www.beckmancoulter.com/wsrportal/bibliography?docname=Bulletin_9231.pdf.
14. Pasricha S, Frazer D, Bowden D, et al. Transfusion suppresses erythropoiesis and increases hepcidin in adult patients with β-thalassemia major: a longitudinal study. Blood. 2013;122(1):124-133.
15. Australian Red Cross Blood Service. Blood Component Information: Circular of Information. Melbourne, Australia: Australian Red Cross Blood Service; 2012.