



Letter to the Editor

The Correlation Between Ineffective Erythropoiesis Biomarkers and Development of Extramedullary Hematopoiesis in Patients with Thalassemia

Keywords: Ineffective erythropoiesis biomarkers, Thalassemia, Extramedullary erythropoiesis.

Published: July 1, 2022

Received: February 15, 2022

Accepted: June 15, 2022

Citation: Chansai S., Yamsri S., Fucharoen S., Fucharoen G., Teawtrakul N. The correlation between ineffective erythropoiesis biomarkers and development of extramedullary hematopoiesis in patients with thalassemia. *Mediterr J Hematol Infect Dis* 2022, 14(1): e2022052, DOI: <http://dx.doi.org/10.4084/MJHID.2022.052>

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To the editor.

Thalassemia is the most common inherited chronic anemia. The patients suffer from anemia resulting from shortened red blood cell (RBC) survival and ineffective erythropoiesis (IE). IE is characterized by the premature death of erythroid precursors in bone marrow or apoptosis of matured nucleated erythroid cells. Moreover, IE also plays an important role in the pathophysiology of thalassemia patients. Increased bone marrow hemopoietic activity from IE frequently leads to the development of extramedullary hematopoiesis (EMH). Nevertheless, IE is believed to be the major mechanism that promotes the development of EMH in patients with thalassemia.¹

Phosphatidylserine (PS) is a negatively charged phospholipid located on inner-cell membranes. Excessive globin chains in thalassemic patients can be accumulated and precipitated within RBC membranes leading to a flip-out of the PS phospholipid to the outer RBC membranes. Exposure of the PS phospholipid on the outer RBC membranes results in RBC destruction at an early stage in the bone marrow.² In thalassemia disease, it is well established that increased PS-exposed RBC levels are associated with pulmonary hypertension (PHT), particularly in splenectomized patients.³ The correlation of highly PS-exposed RBCs and other complications in thalassemia, however, remains to be elucidated.

Growth differentiation factor-15 (GDF15) is one of the markers of ineffective erythropoiesis. It is a regulator of hepcidin expression. In thalassemia, iron overload and ineffective erythropoiesis induce the release of GDF15, leading to a high GDF15 level. Increased GDF15 levels in these patients decrease iron overload by increasing intestinal iron absorption.⁴ High GDF15 levels also correlate with clinical severity in transfusion-dependent thalassemia.⁵

The soluble transferrin receptor (sTfR) is generated during erythroid cell maturation. In thalassemia,

increased sTfR indicates biomarkers of the organs' erythropoietic activity and iron status.⁶ Previous studies demonstrated that the correlation of sTfR and EMH might predict the presence of EMH, particularly in thalassemia patients with intact spleens.⁷

This study aims to evaluate the correlation between ineffective erythropoiesis biomarkers and the development of EMH in patients with thalassemia. It can then be hypothesized that these results could utilize these biomarkers for predicting the risk of developing EMH.

Methods. Ethical approval was obtained from the Institution Review Board (IRB) for human research at Khon Kaen University, Thailand (HE611361). PS-exposed RBCs, GDF15, and TfR levels were evaluated before these thalassemia patients aged >18 who complied with informed consent received blood transfusion therapy. The study was conducted from April 2019 to January 2020 at Srinagarind Hospital, Khon Kaen University, Thailand. The history of RBC transfusions, splenectomy, and laboratory data was reviewed. Spleen length was evaluated by ultrasound technique. Liver iron concentrations (LIC) and cardiac iron concentrations were evaluated by the MRI-T2* technique. EMH was confirmed or excluded by imaging that included ultrasonography, computed tomography (CT) scan, or magnetic resonance imaging (MRI).

Ineffective erythropoiesis biomarkers. PS-exposed RBCs were determined by the flow cytometry technique. RBCs staining and PS exposure were performed as described by Pattanapanyasat *et al.*⁸ Fixed RBCs were measured using FACSCanto II flow cytometry and analyzed with the BD FACSDiva version 6.1.3 software (BD Biosciences). The number of positive cells labeled with FITC-annexin V and PE-glycophorin A was computed. Isotype control-positive

cells were restricted to < 0.3%. GDF15 and sTfR levels were determined using enzyme-linked immunosorbent assay (ELISA) kits, i.e., the GDF-15 Human ELISA kit (Abcam, Cambridge, UK), and the Human sTfR ELISA (BioVendor, Brno, Czech Republic).

Thalassemia genotypes. Hemoglobin and DNA analyses were performed in all patients to determine the thalassemia genotypes. As described previously, common β -thalassemia and α -thalassemia mutations were detected by multiplex-gap PCR and allele-specific PCR assays.⁹

Transfusion requirements. Transfusion-dependent thalassemia (TDT) is a group of patients requiring a regular blood transfusion at less than six-week intervals. The remaining patients were classified as non-transfusion-dependent thalassemia (NTDT).

Statistical analysis. Independent sample Student's t-tests and the Mann-Whitney U-test were used to compare continuous data between two groups. Bivariate correlation analysis was performed with Pearson or Spearman correlations. A P-value < 0.05 was considered statistically significant. Logistic regression methods were used to demonstrate the associations between ineffective erythropoiesis biomarkers and EMH. The receiver operating characteristic (ROC) curves were

constructed to determine the diagnostic performance of ineffective erythropoiesis biomarkers to predict the development of EMH. Data analyses were performed using SPSS 26.0 software (IBM., IL, USA) and STATA 10 statistical software (Stata Corp, College Station, TXP).

Results. One hundred and thirty-one patients were enrolled in this cohort. The patients were classified into two groups: β -thalassemia and α -thalassemia. The clinical characteristics and laboratory data are summarized in **Table 1**. The proportion of patients with splenectomy was more prevalent in patients with β -thalassemia than those with α -thalassemia (51.1% vs. 25.6%, $p = 0.005$). More than half of the patients with β -thalassemia were TDT (54.5%) in contrast to patients with α -thalassemia, of whom most were NTDT (81.4%). Extramedullary hematopoietic tissues were found in thirty-four patients (26.0%). EMH was more prevalent among patients with β -thalassemia (32, 36.4%) than patients with α -thalassemia (2, 4.7%). Serum ferritin and LIC levels were significantly higher among patients with β -thalassemia compared to patients with α -thalassemia. The mean spleen length in non-splenectomized patients was not different in both groups (15.1 vs. 14.5 cm.).

GDF15 levels and GDF15/sTfR ratios in patients with β -thalassemia were significantly higher than in

Table 1. Clinical characteristics and laboratory data of patients with thalassemia.

Parameters	All patients (n= 131)	β - thalassemia (n= 88)	α -thalassemia (n= 43)	p-value
Age at enrollment (yrs.)	33.4 ± 13.3	33.6 ± 12.4	33.2 ± 15.1	0.87
Extramedullary Hematopoiesis (n, %)	34 (26)	32 (36.4)	2 (4.7)	<0.001
Hb level (g/dL)	7.5 (4.6 – 11.3)	7.3 (4.6 – 10.2)	8.2 (5.5 – 11.3)	<0.001
Serum ferritin (ng/mL)	1060 (145 – 6515)	1260 (145 - 6515)	698 (185 - 4329)	0.006
LIC (mg/g dry weight) (n, %)	12.9 ± 9.6 (120, 91.6)	14.7 ± 9.9 (82, 93.2)	9.0 ± 7.5 (38, 88.4)	0.002
Spleen length (cm.)	15.1 ± 3.1	15.5 ± 3.3	14.5 ± 2.8	0.151
Cardiac T2* (n, %)	38.4 ± 8.0 (119, 90.8)	37.8 ± 8.3 (82, 93.2)	39.9 ± 7.0 (37, 86)	0.182
PS levels (%)	1.2 (0.1 -4.1)	1.2 (0.1 – 4.1)	1.2 (0.1 -4.0)	0.804
GDF15 (pg/ml)	31500 (1219.5 - 160500)	43268.3 (6857.1 - 160500)	15928.6 (1219.5 -60571.4)	<0.001
sTfR (µl/ml)	14.6 (2.2 – 73.0)	14.4 (2.2– 68.2)	20.0 (2.2 – 73.0)	0.191
GDF15/sTfR	2454.6 (56.2 – 36507.5)	2946.7 (322.7 – 36507.5)	862.6 (56.2 – 20546.8)	<0.001
Sex (n,%)				
- Male	53 (40.5)	37 (42)	16 (37.2)	0.600
- Female	78 (59.5)	51 (58)	27 (62.8)	
Splenectomy (n,%)				
- Yes	56 (42.7)	45 (51.1)	11 (25.6)	0.005
- No	75 (57.3)	43 (48.9)	32 (74.4)	
Transfusion requirement (n,%)				
- TDT	56 (42.7)	48 (54.5)	8 (18.6)	<0.001
- NTDT	75 (57.3)	40 (45.5)	35 (81.4)	

Table 2 Multivariate analysis of risk factors for extramedullary hematopoietic tissues in 131 patients with thalassemia.

Variables	AOR	95% CI of AOR	p-value
Age (years)	1.04	1.0 – 1.07	0.026
PS-exposed RBCs (%)	1.71	1.05 – 2.80	0.032
GDF15 (pg/ml)	1.0	0.99 - 1.00	0.217
sTfR (μ l/ml)	0.98	0.95 – 1.01	0.067

Abbreviation: AOR= Adjusted odds ratio, 95% CI = 95% confidence interval.

those with α -thalassemia. On the contrary, PS-exposed RBCs and sTfR levels were not statistically significantly different between the two groups.

A multivariate analysis of these risk factors for EMH was performed, as shown in **Table 2**. It was found that advanced age and PS-exposed RBC levels remained significantly associated with EMH after adjustment for other factors with an adjusted odds ratio of 1.04 (95% CI 1.0 -1.07) $p = 0.026$ and 1.71 (95% CI 1.05- 2.8) $p = 0.032$.

The receiver-operating characteristic (ROC) curve analysis of the PS-exposed RBC levels and EMH was constructed to identify the optimal cut-off point (**Figure 1**). The cut-off level of PS-exposed RBCs derived from the ROC curve in this study was 0.45%. Using this cut-off level, the sensitivity and specificity of PS-exposed RBC prediction of EMH were 94.1% and 80.4%, with an area under ROC of 0.67 (95%CI 0.57-0.78), p -value = 0.002.

Discussion. EMH is one of the main thalassemia-related complications in patients with thalassemia. It is more prevalent in patients with β -thalassemia compared to patients with α -thalassemia. Among the ineffective erythropoiesis biomarkers, PS-exposed RBCs showed a modest correlation with EMH. The levels of PS-exposed RBCs were not different between β -thalassemia and α -thalassemia groups. However, the PS-exposed RBC levels in patients with thalassemia were higher than in normal controls.¹⁰ Previous studies showed significantly elevated levels of PS-exposed RBCs in β -thalassemia/Hb E patients who underwent splenectomy and were associated with pulmonary hypertension.¹¹ This study demonstrated a correlation between PS-exposed RBCs levels and EMH in patients with thalassemia.

Abnormal phosphatidylserine (PS) exposure on the surface of RBCs is considered a principal feature of apoptotic RBC precursors and ineffective erythropoiesis in thalassemia.¹² The PS-exposed RBCs may be one of the biomarkers that represent underlying ineffective erythropoiesis. This study showed that PS-exposed RBCs might be considered a biomarker to predict the development of EMH in patients with thalassemia. As shown in **Figure 1**, the PS-exposed RBC levels of more than 0.45% can be used to predict the outcome of EMH with 94.1% sensitivity and 80.4% specificity.

GDF15 is a transforming growth factor- β (TGF- β)

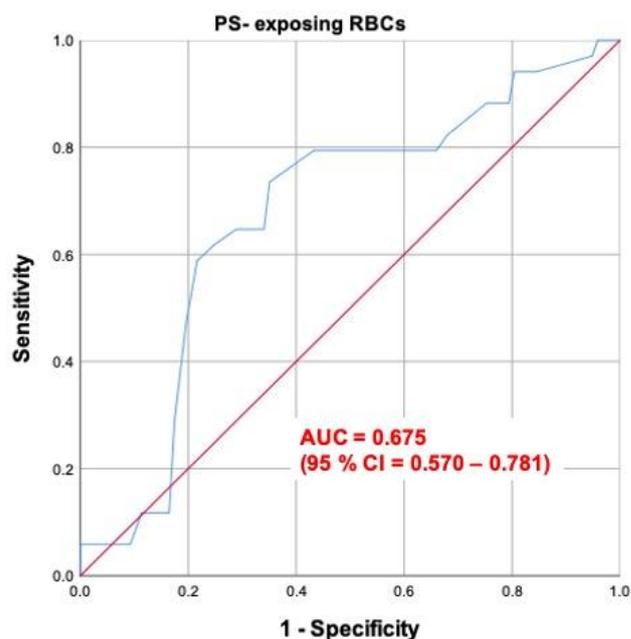


Figure 1. ROC curve of PS-exposed RBC levels and the development of extramedullary hematopoiesis in patients with thalassemia.

superfamily member. Therefore, increased GDF15 levels were considered a marker of ineffective erythropoiesis and iron overload.¹³ This current study showed that GDF15 levels in β -thalassemia were significantly higher than in α -thalassemia, consistent with previous studies.¹⁴ High GDF15 levels suppressed hepcidin expression, contributing to increased gastrointestinal iron absorption and ineffective erythropoiesis.^{4,13} In this study, GDF15 concentration had a weak correlation with EMH. This finding may be explained by ineffective erythropoiesis and the iron overload that the GDF15 levels can influence in patients with thalassemia.

The soluble transferrin receptor is one of the erythropoiesis biomarkers. Previous studies showed that sTfR levels could represent a predictive factor for EMH, particularly in NTDT patients with a spleen.⁷ However, in the present study, sTfR levels between β - and α -thalassemia were not significantly different and could not predict EMH. In addition, the number of β -thalassemia patients with splenectomy was markedly higher than α -thalassemia patients (51.1% vs. 25.6%). This distinction may indicate that splenectomy is a risk factor for paraspinal EMH that supports the hypothesis of an association between iron metabolism and

erythropoiesis expansion and the impact of splenectomy on EMH.⁷ In this study, sTfR levels were correlated with GDF15 levels only in β -thalassemia. A previous study has shown a correlation between log GDF15 and sTfR level in β -thalassemia intermedia (TI) and β -thalassemia/Hb E.¹⁴ Nevertheless, this could be due to a small sample size of α -thalassemia in this study. In addition, most thalassemia patients were non-transfusion-dependent thalassemia (NTDT), and GDF15 levels might affect iron overload and sTfR.

The spleen is the most commonly affected organ in compensating for ineffective erythropoiesis. This study showed that the two groups' spleen length was not significantly different, but massive splenomegaly was different. Massive splenomegaly (spleen length ≥ 17 cm)¹⁵ was more prevalent in patients with β -thalassemia than those patients with α -thalassemia (13 vs. 7 cases). This result demonstrated that an enlarged spleen represented ineffective erythropoiesis in patients with thalassemia.¹⁶ Literature showed that the spleen and liver are the most common sites of EMH in patients with thalassemia.^{17,18}

Extramedullary hematopoiesis is a compensation for the underlying ineffective erythropoiesis. It is a time-dependent process. Advanced age is a significant risk factor for developing EMH in patients with thalassemia. This study also confirms that advanced age is a risk factor for developing EMH.

The limitation of this study is that the number of patients with α -thalassemia in this cohort was relatively small because most of the patients with α -thalassemia were asymptomatic. Therefore, α -thalassemia is rarely encountered in a tertiary hospital. Nevertheless, to the best of the current authors' knowledge, this is the first study demonstrating the association between PS-exposed RBCs and EMH in patients with thalassemia.

In conclusion, extramedullary hematopoiesis is more prevalent in patients with β -thalassemia than in patients with α -thalassemia. Advanced age and high PS-exposed RBC levels had a significant association with EMH. Among the ineffective erythropoiesis biomarkers, PS-exposed RBCs showed a modest correlation with EMH. PS-exposed RBCs may prove useful in predicting the development of EMH in patients with thalassemia.

Acknowledgments. The authors would like to thank Emeritus Professor James A. Will, University of Wisconsin-Madison, for help in preparing the manuscript via the publication clinic of Khon Kaen University, Thailand. This study received grant support from the Thailand Research Fund (TRF) Research Team Promotion Grant (RTA) of the Thailand Science Research and Innovation (TSRI), Thailand (Contract ID RTA6280005) and the Faculty of Medicine, Khon Kaen University.

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Competing interests: The authors declare no conflict of Interest.

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References:

1. Teawtrakul N, Chansung K, Sirijerachai C, et al. A Clinical Risk Score for Predicting Paraspinal Extramedullary Hematopoiesis in Patients with Thalassemia: The KKU-EMH Score. *J Med Assoc Thai* 2017; 100 (4):389-95. PMID: 29911832.
2. Schrier SL. Thalassemia: Pathophysiology of Red Cell Changes. *Annu Rev Med* 1994;45:211-218. <https://doi.org/10.1146/annurev.med.45.1.211>
3. Singer ST, Kuypers FA, Styles L, et al. Pulmonary Hypertension in Thalassemia: Association with Platelet Activation and Hypercoagulable State. *Am J Hematol* 2006;81(9):670-675. <https://doi.org/10.1002/ajh.20640>
4. Tanno T, Bhanu NV, Oneal PA, et al. High Levels of GDF15 in Thalassemia Suppress Expression of the Iron Regulatory Protein Hcpidin. *Nat Med* 2007;13(9):1096-1101. <https://doi.org/10.1038/nm1629>
5. Musallam KM, Taher AT, Duca L, et al. Levels of Growth Differentiation Factor-15 Are High and Correlate with Clinical Severity in Transfusion-Independent Patients with β Thalassemia Intermedia. *Blood Cells Mol Dis* 2011;47(4):232-234. <https://doi.org/10.1016/j.bcmd.2011.07.005>
6. Cazzola M, Beguin Y, Bergamaschi G, et al. Soluble Transferrin Receptor as a Potential Determinant of Iron Loading in Congenital Anaemias Due to Ineffective Erythropoiesis. *Br J Haematol* 1999;106(3):752-755. <https://doi.org/10.1046/j.1365-2141.1999.01600.x>
7. Ricchi P, Ammirabile M, Costantini S, et al. A Useful Relationship between the Presence of Extramedullary Erythropoiesis and the Level of the Soluble Form of the Transferrin Receptor in a Large Cohort of Adult Patients with Thalassemia Intermedia: A Prospective Study. *Ann Hematol* 2012;91(6):905-909. <https://doi.org/10.1007/s00277-011-1385-y>
8. Pattanapanyasat K, Noolsri E, Fucharoen S, et al. Flow Cytometric Quantitation of Red Blood Cell Vesicles in Thalassemia. *Cytometry B Clin Cytom* 2004;57(1):23-31. <https://doi.org/10.1002/cyto.b.10064>
9. Yamsri S, Sanchaisuriya K, Fucharoen G, et al. Prevention of Severe Thalassemia in Northeast Thailand: 16 Years of Experience at a Single University Center. *Prenat Diagn* 2010;30(6):540-546. <https://doi.org/10.1002/pd.2514>

10. Chansai S, Fucharoen S, Fucharoen G, et al. Elevations of Thrombotic Biomarkers in Hemoglobin H Disease. *Acta Haematol* 2018;139(1):47–51. <https://doi.org/10.1159/000486157>
11. Atichartakarn V, Angchaisuksiri P, Aryurachai K, et al. Relationship between Hypercoagulable State and Erythrocyte Phosphatidylserine Exposure in Splenectomized Haemoglobin E/Beta-Thalassaemic Patients. *Br J Haematol* 2002;118(3):893–898. <https://doi.org/10.1046/j.1365-2141.2002.03711.x>
12. Ibrahim HA, Fouda MI, Yahya RS, et al. Erythrocyte Phosphatidylserine Exposure in β -Thalassemia. *Lab Hematol* 2014;20(2):9–14. <https://doi.org/10.1532/LH96.12016>
13. Kaddah AM, Abdel-Salam A, Farhan MS, et al. Serum Hepcidin as a Diagnostic Marker of Severe Iron Overload in Beta-Thalassemia Major. *Indian J Pediatr* 2017;84(10):745–750. <https://doi.org/10.1007/s12098-017-2375-4>
14. Porter JB, Cappellini MD, Kattamis A, et al. Iron Overload across the Spectrum of Non-Transfusion-Dependent Thalassaemias: Role of Erythropoiesis, Splenectomy and Transfusions. *Br J Haematol* 2017;176(2):288–299. <https://doi.org/10.1111/bjh.14373>
15. Taher A, Isma'eel H and Cappellini MD. Thalassemia Intermedia: Revisited. *Blood Cells Mol Dis* 2006;37(1):12–20. <https://doi.org/10.1016/j.bcmed.2006.04.005>
16. Rivella S. Ineffective Erythropoiesis and Thalassemias. *Curr Opin Hematol* 2009;16(3):187–194. <https://doi.org/10.1097/MOH.0b013e32832990a4>
17. Ricchi P, Meloni A, Spasiano A, et al. Extramedullary Hematopoiesis Is Associated with Lower Cardiac Iron Loading in Chronically Transfused Thalassemia Patients. *Am J Hematol* 2015;90(11):1008–1012. <https://doi.org/10.1002/ajh.24139>
18. Ricchi P, Ammirabile M, Spasiano A, et al. Extramedullary Haematopoiesis Correlates with Genotype and Absence of Cardiac Iron Overload in Polytransfused Adults with Thalassaemia. *Blood Transfus* 2014;12 Suppl 1:s124-130. <https://doi.org/10.2450/2013.0287-12>