

Scientific Letters**Elevated Endogenous Alveolar Carbon Monoxide Concentration in Patients with Transfusion-Dependent Thalassemia and Its Relation with Ineffective Erythropoiesis****Keywords:** Thalassemia; Ineffective erythropoiesis; Carbon monoxide; Soluble transferrin receptor.**Published:** July 01, 2025**Received:** December 09, 2024**Accepted:** May 24, 2025

Citation: Xie Y., Liao G., Zhang H., Zhou Y., Xiao Y., Zhou T., Liu D., Yang B., Wei M., Wei Q., Wei C., Lu L., Wang W., Huang J., Geng Z., Wu H., Luo J., Yin X. Elevated endogenous alveolar carbon monoxide concentration in patients with transfusion-dependent thalassemia and its relation with ineffective erythropoiesis. *Mediterr J Hematol Infect Dis* 2025, 17(1): e2025047, DOI: <http://dx.doi.org/10.4084/MJHID.2025.047>

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by-nc/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

To the editor.

Ineffective erythropoiesis is an outstanding feature of thalassemia major. Its main pathological features are premature apoptosis of erythroid progenitor cells in the bone marrow and shortened life span of mature red blood cells in circulation. Ineffective erythropoiesis could lead to complications such as iron overload and extramedullary hematopoiesis in patients with thalassemia, which seriously affects the quality of life of patients. Cazzola reported that reticulocyte production index, unconjugated bilirubin, and soluble transferrin receptor (sTfR) could be used as potentially useful laboratory measurements in the study of patients with anemia due to ineffective erythropoiesis.¹ However, its examination is an invasive operation that requires the extraction of peripheral blood, and laboratory testing takes a certain amount of time. The determination of endogenous alveolar carbon monoxide (CO) concentration could accurately reflect the degree of red blood cell destruction in the body and dynamically monitor the red blood cell damage in patients.² The rapid CO breath test developed by Levitts could evaluate the endogenous alveolar CO concentration, which is a noninvasive, simple, and easy method.^{3,4} To better evaluate the degree of ineffective erythropoiesis in patients with thalassemia and reduce the risk of related complications, this report presents a retrospective study to explore the correlation between CO concentration and ineffective erythropoiesis-related indicators.

A total of 55 patients with transfusion-dependent thalassemia (TDT) were enrolled in this study. The entry criteria of the thalassemia group were as follows: (1) thalassemia was diagnosed by hemoglobin electrophoresis and DNA analysis; (2) patient was diagnosed as TDT; (3) age ≥ 6 years old and could cooperate with blowing; (4) blowing time from the last blood transfusion time ≥ 7 days. The exclusion criteria were: (1) thalassemia patients with organ failure, such

as heart, liver, lung, and kidney; (2) patients with fever, infection, and other conditions; (3) smokers; (4) female patients during pregnancy and lactation. All patients or their parents (if minors) provided written informed consent. CO concentration was assessed using an automatic device (Model WY-2102, WellYearn Technology Co., Ltd., Shenzhen, China), and the final result was taken as an average value after three times of measurements.

The baseline characteristics of patients are summarized in **Table 1**, consisting of 55 patients with TDT (29 β^0/β^0 , 17 β^+/β^0 , 3 β^+/β^+ , 6 β^0/HbE). The median age was 13 years (range 6–30), and 36(66.4%) patients were male, and 6(10.9%) patients had co-inherited α -thalassemia. Nineteen (34.5%) patients underwent splenectomy. Forty-five (81.8%) patients had transfusions more than twenty times in the past two years, that is, an average of one transfusion per month. Regarding the complications of thalassemia, a total of 49 (89.1%) patients were diagnosed with iron overload. Two (3.6%) patients were diagnosed with diabetes and 9 (16.4%) patients suffered cholecystolithiasis. Other complications, such as hypothyroidism, pulmonary hypertension, and leg ulcers, were not observed in our study.

Our findings indicated that CO concentration was significantly higher in patients with TDT than in normal controls, being nearly twice as long in the latter group (median 3.2 vs. 1.5 ppm, **Table 2**). Patients with TDT showed higher erythropoietic activity compared with controls based on the levels of erythropoietin (EPO) and sTfR. The levels of EPO ($P=0.000$) and sTfR ($P=0.000$) were significantly higher in patients with TDT than in controls.

We employed Spearman's rank correlation analysis to explore the correlations between CO concentration and various markers of ineffective erythropoiesis in patients with TDT (**Figure 1**). In terms of the identified

Table 1. Clinical characteristics of 55 patients with TDT.

Characteristics	Case,n (%)
Sex	
Male	36 (65.5%)
Female	19 (34.5%)
Age	
< 18 years	43 (78.2%)
≥18 years	12 (21.8%)
Genotype	
β ⁰ /β ⁰	23 (46.9%)
β ⁰ /β ⁺	17 (34.7%)
β ⁺ /β ⁺	3 (6.1%)
β ⁰ /HbE	6 (12.3%)
α-thalassemia	
with α-thalassemia	6 (12.3%)
without α-thalassemia	49 (89.1%)
Splenectomy status	
Splenectomized	19 (34.5%)
Non-splenectomized	36 (65.5%)
Times of transfusion in the past two years	
<20	10 (18.2%)
≥20	45 (81.8%)
Serum ferritin (ng/ml)	
<1000	6 (10.9%)
≥1000	49 (89.1%)

correlations, CO concentration showed positive correlations with sTfR ($r=0.311$, $p=0.021$), reticulocyte counts ($r=0.432$, $p=0.001$), and unconjugated bilirubin levels ($r=0.368$, $p=0.006$). Similarly, CO concentrations were positively correlated with fetal hemoglobin (HbF) levels ($r=0.339$, $p=0.011$) and the transfusion intervals ($r=0.285$, $p=0.035$). However, we unexpectedly found that hemoglobin levels in these patients were not correlated with the above markers.

Endogenous CO originates mainly from the heme oxidation that genesis during the hemoglobin degradation that follows natural red blood cell rupture. Sannolo et al.⁵ indicated that the production of endogenous CO increased in patients with ineffective erythropoiesis. Similarly, our data also illustrated that patients with thalassemia have boosted CO concentration, which showed red blood cell destruction

increasing and ineffective erythropoiesis. Blood transfusion could effectively inhibit ineffective erythropoiesis in patients with thalassemia. Our study found that CO concentration positively correlated with transfusion intervals. With the length of time between blowing and the last transfusion being longer, bone marrow suppression enhanced, CO concentration increased, and ineffective erythropoiesis gradually worsened in patients with thalassemia.

sTfR is a biomarker of erythropoiesis, originates mostly from erythroblasts and, to a lesser extent, from reticulocytes.⁶ Circulating sTfR is proportional to erythroid precursor mass.⁷ In the previous studies, it was demonstrated that sTfR level was a good marker for evaluating erythropoietic activity in the bone marrow of thalassemia.^{8,9} Therefore, our study used sTfR as an observation marker to evaluate erythroid expansion. Interestingly, we found that the sTfR levels were correlated positively with CO concentration in patients with thalassemia, which indicated that CO concentration of patients with thalassemia was closely associated with bone marrow erythroid expansion, reflecting ineffective erythropoiesis in patients with thalassemia. In a longitudinal study conducted by James et al.,² no correlation between CO concentration and sTfR level was found, but the small size of the study group and only six patients may explain the result. However, it was observed that the level of CO concentration decreased on the fourth day after blood transfusion, and the level of sTfR decreased accordingly, reflecting an inhibition of erythropoiesis.

In β-thalassemia, ineffective erythropoiesis leads to an unconjugated bilirubin level increase, followed by aggravated red blood cell destruction and an increased number of reticulocytes in the peripheral circulation caused by bone marrow erythroid expansion. Except for sTfR, reticulocyte counts and unbound bilirubin are also believed to be potentially useful laboratory measurements in the study of ineffective erythropoiesis.¹ We observed that the CO concentrations were positively correlated with the reticulocyte counts and unbound bilirubin levels, which would help reflect ineffective erythropoiesis. Similarly, James et al.² also reported that the CO concentration correlated with reticulocyte counts, which supported our findings. The level of HbF in patients with β-thalassemia is higher

Table 2. Baseline characteristics and clinical parameters of patients with TDT and normal controls.

Variable	Controls (n=20)	Thalassemia (n=55)	P
Sex, Female	40.0%	34.5%	0.784
Median age, years	15 (10-31)	13 (6-30)	0.069
Median hemoglobin, g/L	141 (122-164)	83 (36-104)	0.000
CO concentration, ppm	1.50 (0.97-2.07)	3.20 (1.00-6.20)	0.000
sTfR, mg/L	0.75 (0.56-3.02)	3.63 (1.09-12.93)	0.000
EPO, mIU/mL	9.66(4.85-24.40)	138.31 (7.05-4160.33)	0.000

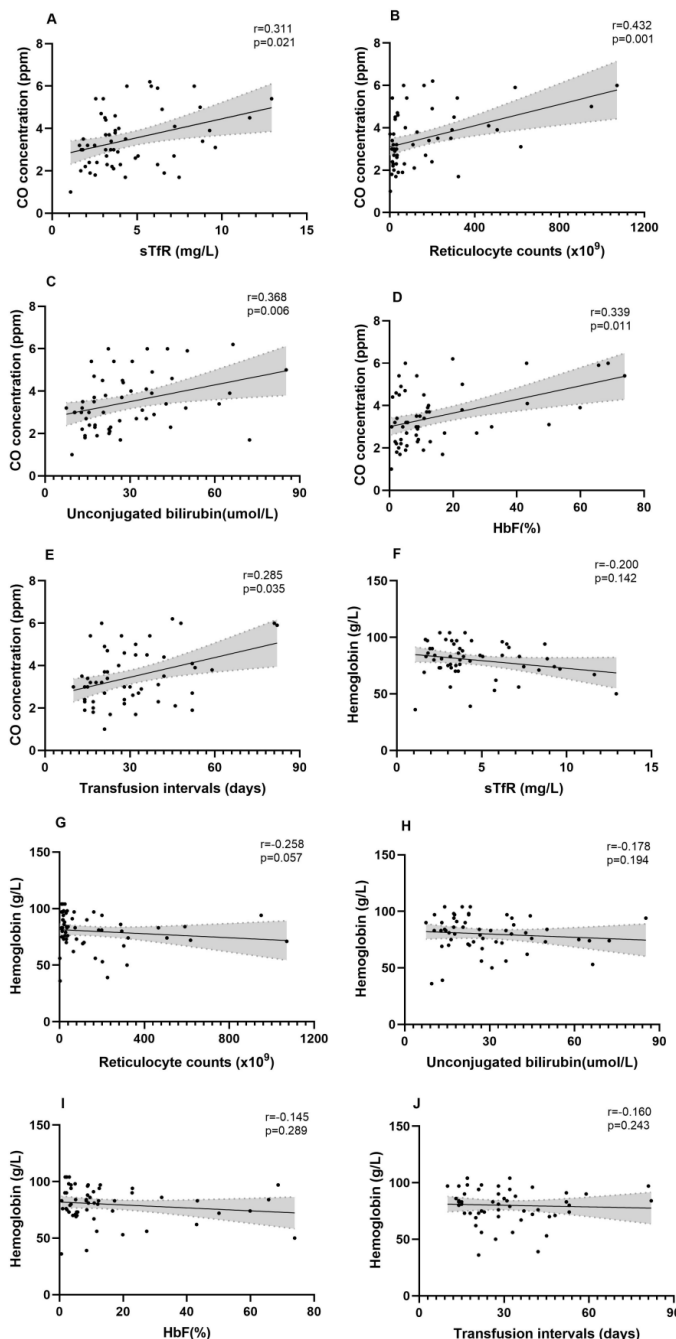


Figure 1. CO concentrations and hemoglobin in different erythropoietic markers in patients with TDT.

than that of normal people.¹⁰ Blood transfusion therapy effectively inhibited ineffective erythropoiesis, and patients with β -thalassemia showed decreased HbF levels.¹¹ Our study showed that CO concentration is positively correlated with HbF levels, reflecting ineffective erythropoiesis and blood transfusion therapy could inhibit ineffective erythropoiesis and reduce CO production. These data suggested that CO concentration could effectively evaluate ineffective erythropoiesis.

Conventionally, studies on the efficacy of thalassemia treatments have focused on changes in hemoglobin levels. However, the current results suggest that compared with hemoglobin, CO concentration is closely related to the ineffective erythropoiesis-related indicators of patients with TDT, indicating its potential value for evaluating the severity and therapeutic effect of β -thalassemia. Therefore, CO concentration determined in this way could thus be a useful indicator for evaluating patient condition and treatment efficacy in patients with β -thalassemia.

In summary, as a noninvasive and rapid examination method, the CO breath test could better reflect the ineffective erythropoiesis in patients with thalassemia. This would benefit clinicians by helping them better manage patients with TDT and provide better individualized clinical treatment decisions for them.

Ethics approval and consent to participate. All procedures were carried out according to the relevant guidelines. This study was approved by the ethics committee of the 923rd Hospital of the Joint Logistics Support Force of the People's Liberation Army. The patients or their parents involved in this study provided informed consent.

Acknowledgments. We want to thank all participants involved in this study. This study was supported by the Scientific Research Project of Guangxi Zhuang Autonomous Region Health Committee (Z-A20231086) and the Scientific Research Fund Project of Guangzhou City Life Oasis Public Welfare Service Center (GZLZ-HEMA-008).

Yanni Xie^{1*}, Guiping Liao^{1*}, Hongyuan Zhang^{2*}, Yali Zhou¹, Yunshuo Xiao¹, Tianhong Zhou¹, Dongmei Liu¹, Beibei Yang¹, Manlv Wei¹, Qiuying Wei¹, Changqing Wei¹, Lina Lu¹, Weiren Wang¹, Jian Huang¹, Zhili Geng¹, Hui Wu¹, Jingting Luo³, Xiaolin Yin¹.

¹ Department of Hematology, the 923rd Hospital of the Joint Logistics Support Force of the People's Liberation Army.

² Department of Equipment, the 923rd Hospital of the Joint Logistics Support Force of the People's Liberation Army.

³ Guangdong Breath Test Engineering Technology Research Center, College of Optoelectronic Engineering, Shenzhen University.

* These authors equally contributed to this work.

Competing interests: The authors declare no conflict of Interest.

Correspondence to: Jingting Luo and Xiaolin Yin. E-mail: 280662268@qq.com

References:

1. Cazzola M. Ineffective erythropoiesis and its treatment[J]. *Blood*, 2022, 139(16):2460-2470.
<https://doi.org/10.1182/blood.2021011045>
PMid:34932791
2. Ellen Butensky J, Hendrik J V, Ronald J W, David K S, Elliott V, Laurie S, Judith Y H, Julie S, Daniel W G Paul H. Elevated exhaled carbon monoxide concentration in hemoglobinopathies and its relation to red blood cell transfusion therapy[J]. *Pediatr Hematol Oncol*, 2010, 27(2):112-121.
<https://doi.org/10.3109/08880010903536227>
PMid:20201692
3. Zhang H, Ma Y, Liu Q, Ye T, Meng F, Zhou Y, Yu G, Yang J, Jiang H, Wang Q, Li G, Ji Y, Zhu G, Du L Ji K. Human erythrocyte lifespan measured by Levitt's CO breath test with newly developed automatic instrument[J]. *J Breath Res*, 2018, 12(3):036003
<https://doi.org/10.1088/1752-7163/aaaf1>
PMid:29400658
4. Zhou Y., Li J., Wei M., Lu L., Luo J., Jin X., Yang S., Yang L., Liao G., Zhou T., Huang J., Chen Y., Yin X. Measurement of erythrocyte lifespan using a CO breath test in patients with thalassemia and the impact of treatment. *Mediterr J Hematol Infect Dis* 2023, 15(1): e2023050
<https://doi.org/10.4084/MJHID.2023.050>
PMid:37705519 PMCID:PMC10497307
5. Sannolo N, Farina V Fiorillo A. Abnormal endogenous carbon monoxide production in children with ineffective erythropoiesis[J]. *Ann Clin Biochem*, 1992, 29(Pt 4):397-399.
<https://doi.org/10.1177/000456329202900404>
PMid:1642444
6. Baynes R, Shih Y Cook J. Mechanism of production of the serum transferrin receptor[J]. *Adv Exp Med Biol*, 1994, 356:61-68.
https://doi.org/10.1007/978-1-4615-2554-7_7
PMid:7534031
7. Cazzola M, Guarnone R, Cerani P, Centenara E, Rovati A Beguin Y. Red blood cell precursor mass as an independent determinant of serum erythropoietin level[J]. *Blood*, 1998, 91(6):2139-2145.
<https://doi.org/10.1182/blood.V91.6.2139>
PMid:9490701
8. Aysin D, Nese Y, Tunc F, Feride D Abdurrahman K. Serum transferrin receptor levels in beta-thalassemia trait[J]. *J Trop Pediatr*, 2004, 50(6):369-371.
<https://doi.org/10.1093/tropej/50.6.369>
PMid:15537726
9. Christophille S, Ioannis P, Joanne T-S, Helene S, Vassilios L, Anna M-M, Alexandra S Emmanuel K. Erythroid bone marrow activity and red cell hemoglobinization in iron sufficient beta-thalassemia heterozygotes as reflected by soluble transferrin receptor and reticulocyte hemoglobin in content. Correlation with genotypes and Hb A(2) levels[J]. *Haematologica*, 2003, 88(6):631-636.
10. Origa R. beta-Thalassemia[J]. *Genet Med*, 2017, 19(6):609-619.
<https://doi.org/10.1038/gim.2016.173>
PMid:27811859
11. Winichagoon P, Fucharoen S, Chen P Wasi P. Genetic factors affecting clinical severity in beta-thalassemia syndromes[J]. *J Pediatr Hematol Oncol*, 2000, 22(6):573-580.
<https://doi.org/10.1097/00043426-200011000-00026>
PMid:11132233