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Scientific Letter

Measurement of Erythrocyte Lifespan Using a CO Breath Test in Patients with Thalassemia and the Impact of Treatment

Keywords: Erythrocyte lifespan; Thalassemia; End-expiratory CO concentration; Splenectomy.

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To the editor.

Thalassemia comprises a diverse group of genetic disorders that affects the synthesis of globin chains, with a global distribution.[1] The type of thalassemia depends on the defective globin chain, with α-thalassemia and β-thalassemia being the most clinically important forms.[2] From a clinical perspective, thalassemia can be classified according to the severity of the anemia and the need for regular red blood cell (RBC) transfusions. Mild thalassemia is caused by the heterozygous inheritance of one thalassemia mutation and presents clinically as minimal, microcytic, and hypochromic anemia. Patients with severe thalassemia require regular lifelong transfusions of RBCs from early childhood, while moderate thalassemia is associated with less severe anemia that does not require blood transfusions or sporadic transfusions. However, some patients with thalassemia intermedia may eventually benefit from regular blood transfusions, while patients with thalassemia major may discontinue transfusions after splenectomy.[1,3]

An imbalance in globin chain production in thalassemia leads to erythrocyte skeleton damage and cell dysfunction, resulting in a shortened lifespan. Erythrocyte lifespan (ELS) indicates the survival time of RBCs in the blood circulation and is the most direct and reliable indicator of the degree of RBC destruction.

Methods for determining ELS fall into two general categories, namely radioactive or nonradioactive labeling and CO breath tests. Conventional methods of ELS detection have disadvantages such as radiation hazards, allergy risk, and cumbersome operation, which make them unsuitable for large-scale detection and application in clinical practice. The ^{15}N-glycine labeling technique is the gold standard method for determining ELS; however, although this is an accurate technique for measuring ELS that avoids safety issues related to radioactivity or possible allergic reactions, its usefulness in clinical settings is seriously hindered by the fact that it takes several weeks to complete the analyses.[4,5] In contrast, Levitt's CO breath test also provides a reliable technique for determining ELS and has a simpler protocol with faster results, making it more useful in clinical applications.[6] Furthermore, the CO breath test performs as well as the ^{15}N-glycine labelling technique for distinguishing hemolysis.[7]

The current study aimed to investigate the use of Levitt's CO breath test as a quantitative measure of ELS, to explore the feasibility of using ELS as an indicator for determining the severity of different types of thalassemia, and to assess the impact of treatment.

Materials and Methods. A total of 209 patients with thalassemia were enrolled from the 923rd Hospital of the Joint Logistics Support Force of the People's Liberation Army between March 2022 and May 2023. Thalassemia syndromes can be classified phenotypically into non-transfusion-dependent thalassemia (NTDT) (n=70), or transfusion-dependent thalassemia (TDT) (n=139) based on whether blood transfusions are required for long-term survival. Additionally, patients with thalassemia were subsequently classified into four types based on their clinical manifestations and genotype: thalassemia major (TM, n=83), thalassemia intermedia (TI, n=46), hemoglobin H (HbH, n=63), and α-thalassemia co-inherited with β-thalassemia (Mix, n=17). HbH disease was further subcategorized into deletional HbH (del-HbH, n=12) and non-deletional types (all with HbH Constant Spring, HbH-CS, n=51).

This study was approved by the ethics committee of the 923rd Hospital of the Joint Logistics Support Force of the People's Liberation Army, and all participants provided written informed consent. For participants under 14 years of age, their guardians signed the informed consent form on their behalf.

Hemoglobin levels were analyzed using a Bio-Rad Variant II high-performance liquid chromatography system (Hercules, CA, USA). CO samples were

collected by the breath analyzer (Model WY-2101,WellYearn Technology Co., Ltd., Shenzhen, China), the measured CO was calculated according to the formula in the literature to obtain the RBCLs of the subject, which is calculated by Levitt's formula: ELS=(1.38×[Hb])/PCO where [Hb] is the hemoglobin concentration of patients in g/L, PCO is the endogenous alveolar CO concentration in ppm (parts per million, mg/L).[6,8]

Statistical analysis. Statistical analysis was performed using SPSS 26.0, and data were presented as median and range. Numerical variables were compared between groups using Student's *t*-test or the Mann-Whitney rank-sum test. Correlation analysis was carried out using Spearman's rank correlation coefficient. *P* values <0.05 were considered statistically significant.

Results.

CO concentrations and ELS values in different types of thalassemia. The endogenous alveolar CO concentration and ELS were compared between patients with different types of thalassemia. Patients with TI had the highest CO concentration [7.2 ppm (3.9–22)] compared with patients with TM [5.7 ppm (2.1–18.1)] (*P*<0.001), HbH-CS [6.0 ppm (2.2–12.7)] (*P*=0.023), del-HbH [4.05 ppm (1.4–13.6)] (*P*<0.001), and Mix [5.1 ppm (2.2–11.7)] (*P*=0.004) (**Figure 1A**). The CO concentration was significantly higher in patients with HbH-CS compared with patients with del-HbH (*P*=0.011) (**Figure 1A**). In contrast, the ELS was significantly lower in patients with TI [16.5 ppm (6–30)] compared with patients with TM [22 days (7–68)] (*P*<0.001), HbH-CS [22 days (6–72)] (*P*=0.025), del-HbH [32.5 days (11–72)] (*P*<0.001), and Mix [23 days (6–61)] (*P*=0.002) (**Figure 1B**). In addition, the ELS was significantly higher in patients with del-HbH compared with patients with HbH-CS (*P*=0.030) (**Figure 1B**). However, there was no difference in CO concentration [6.05 ppm (1.4–16.8) vs. 5.8 ppm (2.1–22.0), *P*=0.208] and ELS [19 days (6–72) vs. 20 days (6–68), *P*=0.250] between NTDT and TDT.

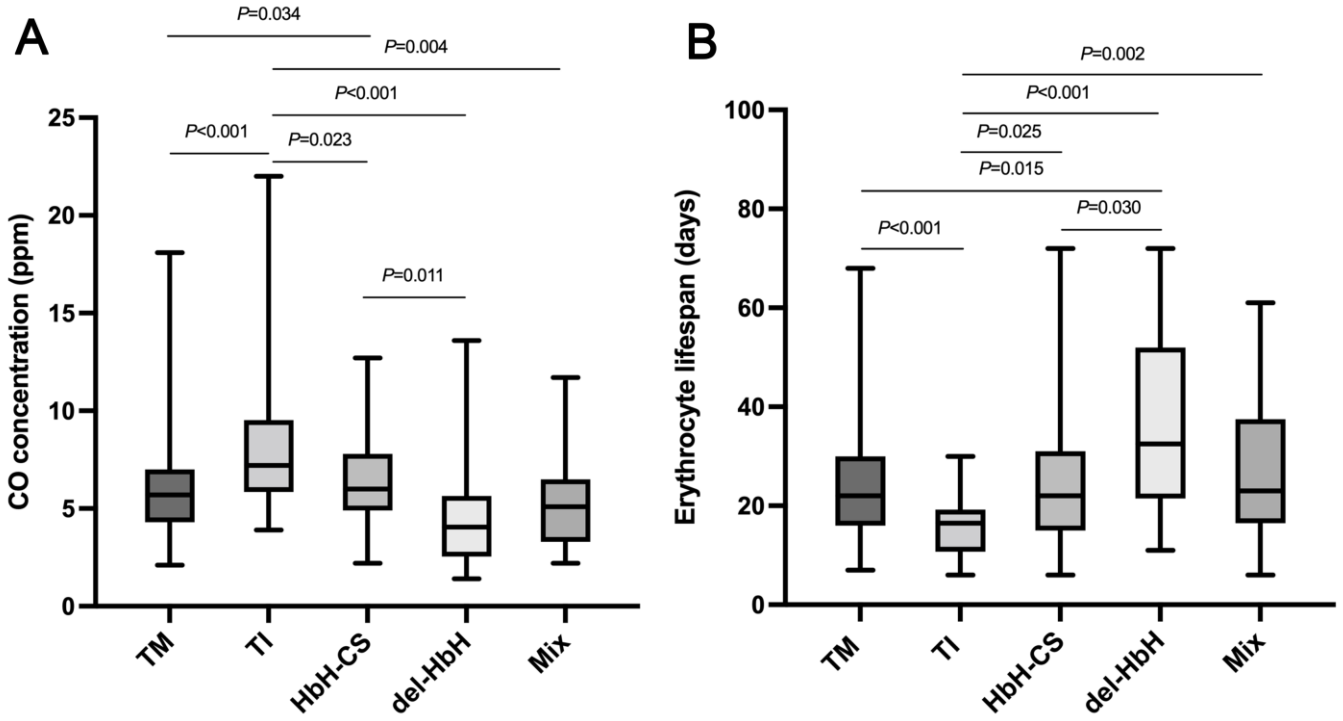

Figure 1. Comparison of CO concentrations and RBCs in patients with different types of thalassemia.

Effects of splenectomy on different types of thalassemia. Few patients had del-HbH or Mix, and we therefore compared the effects of splenectomy among patients with TM, TI, and HbH-CS. There was no significant difference in CO concentration or ELS between patients with TM and TI who underwent splenectomy compared to those who did not (**Figure 2A-D**). However, patients with HbH-CS who underwent splenectomy had significantly lower CO levels (*P*=0.01) (**Figure 2E**) and higher ELS than patients with HbH-CS without splenectomy (*P*=0.02) (**Figure 2F**). Furthermore, there was no significant difference in CO concentration or ELS between patients with NTDT and TDT who underwent splenectomy compared to those who did not (*P*>0.05).

Effects of hemoglobin levels and transfusion in patients with different types of thalassemia. Spearman's correlation analysis showed that hemoglobin levels were negatively correlated with CO concentration (*r*=-0.158, *P*=0.023) (**Figure 3A**) and positively correlated with ELS (*r*=0.535, *P*<0.001) (**Figure 3B**). To investigate the

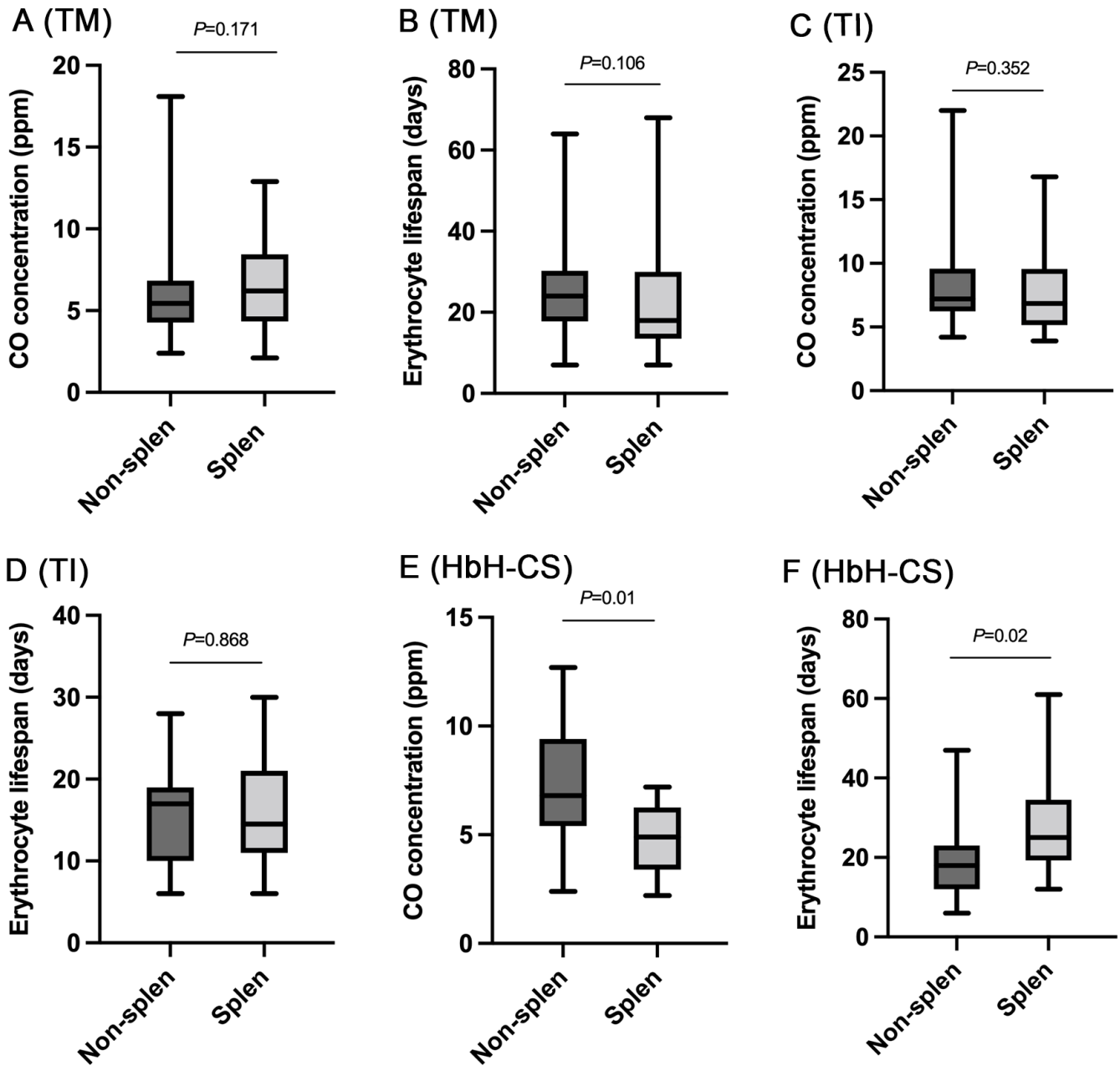

Figure 2. Effects of splenectomy in patients with different types of thalassemia.

association between the number of transfusions or transfusion interval and CO concentration or ELS, separate studies were conducted in patients with TDT. As a result, patients with more than 10 transfusions in the last year had a lower CO concentration [4.9 ppm (2.1–12.9) vs. 7.05 ppm (3.3–22.0), $P<0.001$] and higher ELS [27 days (8–68) vs. 17 days (6–28), $P<0.001$] than patients with fewer than 10 transfusions. In addition, the length of time between blowing and the last transfusion in patients with TDT was positively correlated with CO concentration ($r=0.209$, $P=0.014$) (**Figure 3C**) and negatively correlated with ELS ($r=-0.267$, $P=0.001$) (**Figure 3D**). However, no association was found in patients with NTDT.

Discussion. For simpler and better management, thalassemia is currently divided into NTDT and TDT based on whether patients rely on blood transfusions for long-term survival. Traditionally, thalassemia phenotypes can be divided into carrier, mild, intermediate, and severe. Carriers and patients with mild disease are usually asymptomatic and do not require treatment. In contrast, patients with the most severe type of α-thalassemia, Hb Bart's hydrops fetalis syndrome, usually die very early. The main types of thalassemia that require clinical attention are thus TM, TI, and HbH disease.[9] The current study compared ELS values among different types of thalassemia and used them to validate the role of splenectomy.

In this study, hemoglobin levels were similar in patients with various types of thalassemia, but the expiratory CO concentration was highest in patients

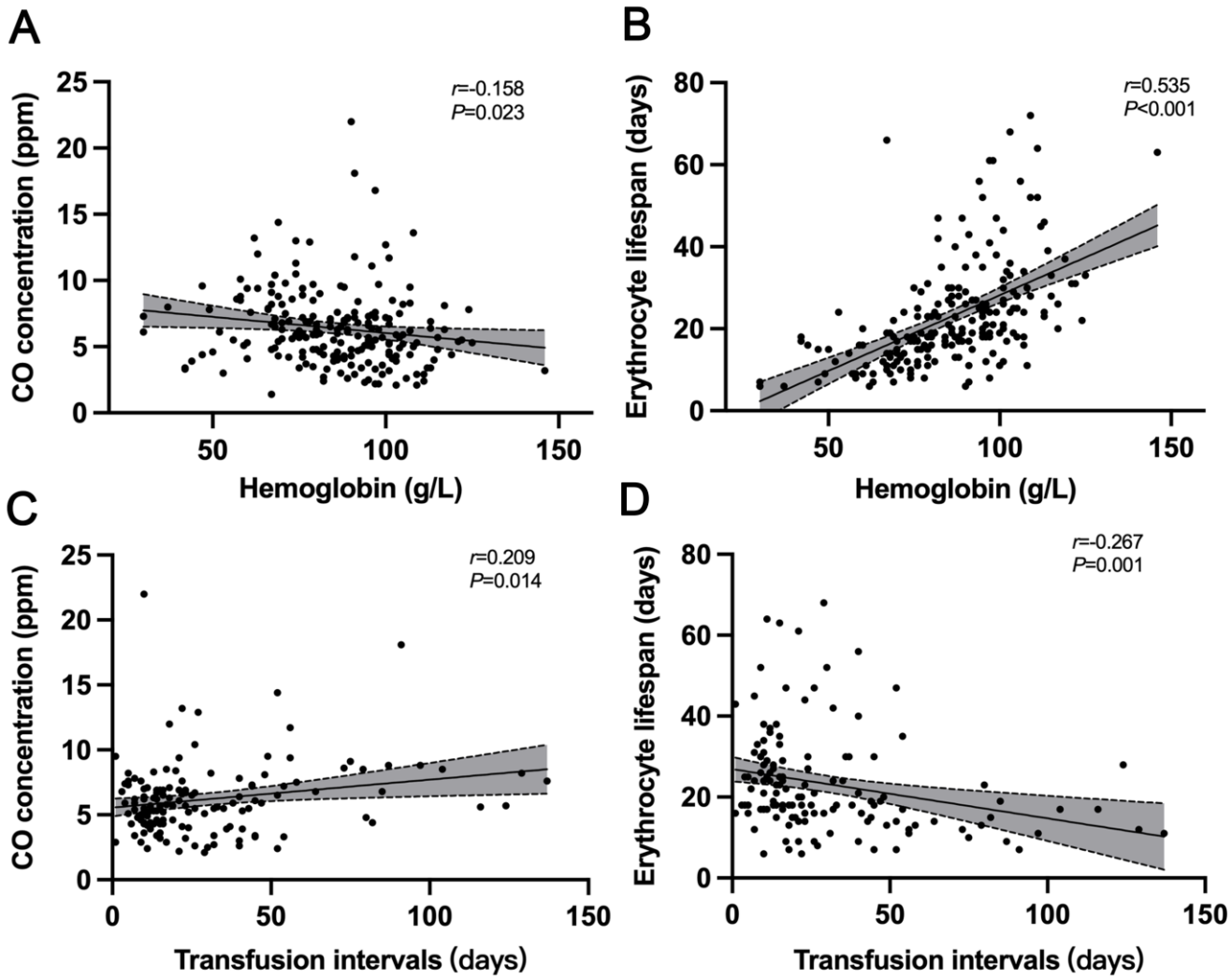

Figure 3. Plots of hemoglobin levels or transfusion intervals versus CO concentration or ELS.

with TI, followed by HbH-CS, TM, and Mix, and lowest in del-HbH disease. ELS showed the opposite pattern, being shortest in patients with TI, followed by HbH-CS, TM, and MIX, and longest in del-HbH disease. Li et al.[10] reported that the ELS values in patients with mild β-thalassemia and severe α/β thalassemia were 67.5 days and 30.2 days, respectively, but did not provide definitions of severe and mild β-thalassemia or clarify the type of α-thalassemia. HbH disease can be classified as deletional or non-deletional based on genotype. HbH-CS accounts for most cases of non-deleted HbH in Guangxi, China, and its clinical manifestations are more severe than del-HbH.[11] Among patients with HbH, ELS was shorter in patients with HbH-CS than in patients with del-HbH, although the severity of anemia was similar in both groups. This also reflected the more severe hemolysis in patients with non-deletional compared with deletional HbH disease.

Patients with TI had a lower ELS than patients with TM, possibly because patients with TM had more blood transfusions. Patients with TM had a shorter interval from the last transfusion to insufflation and a higher frequency of transfusions in the last year compared with patients with TI. The average lifespan of transfused RBCs is higher than that in patients with thalassemia.[12] In addition, blood transfusions can also inhibit hematopoiesis in thalassemia patients, reduce ineffective hematopoiesis, and reduce the corresponding RBC destruction.[13,14] In the present study, transfusion intervals were positively correlated with ELS in patients with TDT. Previous studies also reported a higher incidence of patients with erythroferrone, an indicator of ineffective hematopoiesis, among TI compared with TM patients.[15,16] This is consistent with clinical observations, in that patients with TM with standardized blood transfusion have fewer complications than patients with TI.[17] In addition, patients with TI can be inadequately treated if hemoglobin values alone are used to guide blood transfusion. All the patients with Mix included in this study were β-globin gene double heterozygotes with one or two α-globin gene mutations, largely associated with TI. In patients with β-thalassemia, co-inheritance of α-globin gene variants, leading to absence or reduction of α-globin synthesis, were associated with a milder clinical course.[18] In our cohort, ELS was significantly higher in patients with

Mix and significantly higher than that in patients with TI, which further confirmed that co-inheritance of α-thalassemia alleviated hemolysis in patients with β-thalassemia. However, the CO concentration and ELS in patients with Mix were more similar to those in patients with TM. Overall, ELS or CO concentration thus better reflect the severity of different types of thalassemia compared with hemoglobin.

Splenectomy is a therapeutic option for thalassemia. The current study found no significant difference in CO concentrations or ELS in relation to splenectomy in patients with either TM or TI. However, splenectomy reduced the CO concentration and prolonged ELS in patients with HbH-CS, suggesting that splenectomy reduced RBC destruction in patients with α-thalassemia rather than β-thalassemia. We also previously observed that splenectomy reduced blood transfusions and increased hemoglobin in patients with HbH.[19] This is mainly due to the different sites of RBC destruction, the former being mainly in the spleen and the latter mainly in the bone marrow.[20] There was no significant difference in hemoglobin levels between HbH-CS patients with and without splenectomy, but the difference in ELS was significant, suggesting that ELS was a more sensitive indicator than hemoglobin, as confirmed in clinical trials of other drugs.[21]

This study had certain limitations. First, the sample sizes were small. Although there was a total of 209 cases of thalassemia, after dividing the patients into five groups, the size of each group was relatively small, especially in the case of del-HbH disease, which only included 12 cases. Second, the sample was not representative. All the data were from patients presenting at outpatient or inpatient visits, there was selection bias, and only patients with relatively severe disease generally came to hospital, and the proportions of patients with different types of thalassemia were therefore not consistent with the distribution of large sample surveys. The results of this study should thus be interpreted with caution. Notably, however, the findings reflected the actual situation of clinical patients.

Conclusions. In conclusion, our observations suggested that there were large differences in CO concentrations and ELS values among patients with different types of thalassemia. Measuring ELS will provide more information for assessing the severity of thalassemia and judging the effects of blood transfusions and treatments, especially in clinical drug trials.

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