Pre- and Post-transfusion Complement Activation in Transfusion-dependent β-thalassaemia

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Dear Editor,

Thalassaemia is a common genetic disorder that has been under detailed investigation over the past 40 years. Introduction of safe transfusion and supportive practices has significantly improved survival of transfusion-dependent β-thalassaemia (TDT).1 Although regular red blood cell (RBC) transfusions remain the mainstay of treatment, their role in the pathophysiology of complications in TDT is unclear.

Activated peripheral blood and endothelial cells along with platelet and erythrocyte microparticles have been detected in thalassaemia patients, especially post-splenectomy.2,3 Microparticles have been also found in stored RBCs. Microparticles interact with the complement system generating terminal complement activation products, such as C5b-9 (also known as the membrane attack complex), in whole blood and nonleukocyte-reduced RBC components.4 Stored RBCs are also susceptible to complement degradation. Furthermore, hemolysis during RBC storage and processing could contribute to complement activation.5 Therefore, transfusions might contribute to a cycle of thrombosis and complement activation.

Nevertheless, limited data exist on complement activation in TDT. The link between hemolysis and complement activation in thalassaemia may be explained not only by free heme that acts as a C5 convertase but also by decreased expression of complement regulators found in RBCs of TDT patients.6 Chapin and colleagues have recently reported unpublished data of increased levels of C5b-9 in TDT patients that decreased post-transfusion.7 However, the role of disease characteristics and transfusion products remains unclear. Therefore, we aimed to determine whether increased complement activation is evident in TDT; whether it is associated with disease characteristics and whether it is exacerbated post transfusion.

Consecutive TDT patients were enrolled prospectively for 8 weeks (November to December 2017). Our study was approved by the Institutional Review Board and conducted in accordance with the Declaration of Helsinki. Patient history and clinical and laboratory data were recorded. Sera were collected immediately before transfusion and 1 hour after completion of transfusion in each patient and stored at –80°C. Complement activation was detected in patient sera measuring soluble human C5b-9 with a commercially available ELISA kit (AMSbio, Abingdon, UK). The assay has a sensitivity of 1 pg/mL and coefficient of variability of 4.4%. Normal human sera from 10 age and gender-matched Caucasian healthy volunteers were used as negative controls and lipopolysaccharides (1 mg/mL, Sigma, St Louis, MO)-incubated normal sera as positive controls, based on previously reported experiments.8

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 20.0 for Windows (SPSS, Chicago, IL). Results are presented for continuous variables as mean± standard deviation or (for skewed variables) as median±interquartile range and for qualitative variables as frequencies. Statistical analyses were carried out using the independent samples Student t test or the Mann-Whitney U test to compare differences between groups. The Wilcoxon signed-rank test was performed to compare paired pre- and post-transfusion values. Pearson or Spearman correlation coefficients were used according to the variable's distribution. A P value ≤0.05 was considered statistically significant.

We studied 43 TDT patients (45.5±25.6 years of age, 21 males: 24 females). In our unit, all patients systematically receive phenotypically matched RBCs stored for <10 days. RBCs are stored using ACPDA1 (citrate, phosphate, glucose, and adenine) as a preservative solution. Leukodepleted RBCs are provided by...
high-efficient leukoreduction filter sets 2 to 4 hours before transfusion (Leucolab LCG4, Macopharma, France). Donor and recipient are prospectively matched for ABO Rh (CcEe), Kell and the absence of specific antigens in patients with alloantibodies. All patients in the present study receive 2 RBC units every 2 weeks with a goal of pretransfusion hemoglobin >9.5g/dL.

Soluble C5b-9 levels before transfusion (median 68.2ng/mL, interquartile range 91.2ng/mL, compared with C5b-9 detected in normal human serum (median interquartile range 62.1ng/mL) were not significantly increased 1 hour post-transfusion (median 85.6ng/mL, interquartile range 72.5ng/mL, P = 0.219). However, C5b-9 levels significantly increased 1 hour post-transfusion compared with pretransfusion levels in thalassaemia intermedia patients (median 94[107] 32[34.5] ng/mL, P = 0.030). As shown in Figure 1, C5b-9 increased in 24 out of 45 patients and was stable or slightly decreased in 21 patients. Given the characteristics of RBC products used in the present study, administration of exogenous C5b-9 was not expected to occur in our patients. Indeed, there was no difference in transfusion products (including storage duration, ABO type and RBC volume/kg) between patients with an increase of C5b-9 post-transfusion compared with patients with stable or decreased C5b-9. However, diagnosis of thalassaemia intermedia, pretransfusion C5b-9 levels, and liver iron concentration were significantly higher in patients with an increase of C5b-9 post-transfusion (Table 1).

Then, we analyzed clinical factors associated with C5b-9 levels post-transfusion. Interestingly, C5b-9 levels post-transfusion were significantly increased in patients traditionally classified as thalassaemia intermedia (P = 0.041) and patients undergone splenectomy (P = 0.034). In addition, both patients with thalassaemia intermedia and patients with splenectomy were older and had significantly higher platelet values compared with patients with thalassaemia major (P = 0.035 and P = 0.021, respectively) and patients without splenectomy (P = 0.008 and P < 0.001, respectively). C5b-9 values did not correlate with other patient characteristics (iron overload, RBC volume/weight, or development of red cell alloantibodies).

Despite the limited studies in the field, Chapin et al recently reported increased soluble C5b-9 levels in patients with TDT that decreased post-transfusion. However, this review article does not provide details on patient characteristics and methods. Therefore, no direct comparison between studies is feasible. Another interesting finding of our study concerns the absence of excess complement activation before transfusion in TDT patients disregarding thalassaemia intermedia or splenectomy. This could be a result of low complement availability or complement dysregulation. The latter is supported by a recent study confirming that RBCs of thalassaemia major patients present with a lower CD55 and CD35 (complement receptor type 1) expression. Lower expression of these important complement

### Table 1

| Characteristics                                      | Increased C5b-9            | Stable or Decreased C5b-9 | P     |
|------------------------------------------------------|---------------------------|---------------------------|-------|
|                                                      | Post-Transfusion (n = 24) | Post-Transfusion (n = 21) |       |
| Age, years (mean ± SD)                               | 38.1 ± 9.7                | 40.1 ± 13.1               | 0.57  |
| Thalassaemia intermedia, n, %                        | 10.41.7%                  | 3.14.3%                   | 0.043 |
| Pretransfusion C5b-9, ng/mL, median (IQR)            | 94 [107]                  | 32 [34.5]                 | 0.030 |
| 1-Year RBC volume/patient’s weight, mL/kg (mean ± SD)| 154.6 ± 55.3             | 159.1 ± 47.8              | 0.77  |
| Platelets, 10^9/L, (mean ± SD)                       | 467.5 ± 216.2             | 410.8 ± 219.6             | 0.870 |
| Ferritin, ng/mL (mean ± SD)                          | 998.5 ± 936.6             | 1074.9 ± 931.5            | 0.773 |
| Splenectomy, n, %                                    | 14.58.3%                  | 8.36.1%                   | 0.175 |
| Erythroblast treatment, n, %                          | 10.41.7%                  | 7.33.3%                   | 0.565 |
| Combined iron chelation treatment, n, %              | 9.43.7%                   | 10.47.6%                  | 0.493 |
| Liver iron concentration, mg/g (mean ± SD)           | 7.04 ± 5.8                | 3.24 ± 3.8                | 0.014 |
| Hepatic T2*, ms (mean ± SD)                          | 13.2 ± 11.1               | 16.7 ± 9.9                | 0.316 |
| Cardiac T2*, ms (mean ± SD)                          | 33.1 ± 5.8                | 29.2 ± 5.9                | 0.078 |
| Red cell alloantibodies, n, %                        | 1.4.2%                    | 3.14.3%                   | 0.234 |

IQR = interquartile range, SD = standard deviation.
regulators could result in susceptibility of RBCs to complement mediated effects that would not necessarily be evident in patient sera. This finding needs to be further studied in larger experimental studies and cohorts.

Our study provides preliminary in vitro results of pre- and post-transfusion complement activation using a robust marker in a real-world patient population. It would be interesting to further study the effects of transfusion at later time-points, for example, 24 hours post-transfusion. This would determine whether the observed increase in complement activation is transient or longer lasting. More importantly, the use of robust complement activation markers is necessary to obtain comparable results among studies.

In conclusion, the effect of blood transfusions on complement activation has been scarcely investigated in TDT patients and seems controversial. We found an increase of C5b-9 levels in a subgroup of patients originally classified as thalassaemia intermedia and in patients with splenectomy. These groups of patients have been traditionally considered to be of high thrombotic risk. The interplay between complement and thrombosis in these patients and the role of transfusions need to be further investigated in future studies.

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