

Splenectomy and Complement Regulation in Erythrocytes: Implications for Hemolysis in Pediatric β -Thalassemia Major

Amal Mohammed Bakr¹, Mervat Atfy Mohammed¹, Diana Hanna Abdelmalek¹, Weaam Ibrahim Ismail^{2*}

¹ Pediatrics Department, Faculty of Medicine, Zagazig University, Egypt.

² Clinical Pathology Department, Faculty of Medicine, Zagazig University, Egypt.

Corresponding Author

Amal Mohammed Bakr

Pediatrics Department, Faculty of Medicine, Zagazig University, Egypt.

Email: a.bakr22@medicine.zu.edu.eg.

Cite this paper as: Amal Mohammed Bakr, Mervat Atfy Mohammed, Diana Hanna Abdelmalek, Weaam Ibrahim Ismail (2025) Splenectomy and Complement Regulation in Erythrocytes: Implications for Hemolysis in Pediatric β -Thalassemia Major. Journal of Neonatal Surgery, 14, (33s) 960-964

Received: 29-12-2024 Reversed: 28-02-2025 Accepted:22-04-2025 Published: 30-04-2025

ABSTRACT

β -Thalassemia major is a hereditary hemoglobinopathy characterized by ineffective erythropoiesis, chronic anemia, and enhanced red blood cell (RBC) destruction. Hemolysis in this disorder results from both intrinsic membrane defects and extrinsic immune-mediated mechanisms. The spleen plays a central role in culling defective erythrocytes and regulating immune responses, including complement activation. Splenectomy is frequently performed in pediatric β -thalassemia major to ameliorate transfusion requirements and reduce extravascular hemolysis. However, removal of the spleen alters key pathways of complement regulation on erythrocyte surfaces, potentially exacerbating hemolytic processes. This review synthesizes current evidence on the interplay between splenic function, complement regulatory proteins (e.g., CD55, CD59), and erythrocyte survival in β -thalassemia major. We examine how splenectomy modifies complement system dynamics, leading to changes in opsonization, membrane attack complex formation, and susceptibility to intravascular hemolysis. Additionally, we discuss emerging biomarkers for complement activation, the impact of chronic transfusion therapy, and the risk of thromboembolic complications post-splenectomy. Understanding these mechanisms deepens insight into the pathophysiology of hemolysis in pediatric β -thalassemia major and highlights potential therapeutic targets to mitigate complement-mediated RBC destruction while optimizing patient outcomes.

Keywords: β -thalassemia major; Hemolysis; CD55; CD59; Pediatric hematology; Ineffective Erythropoiesis; Thromboembolic Complications..

INTRODUCTION

β -Thalassemia major is a severe, transfusion-dependent hemoglobinopathy caused by mutations in the β -globin gene, resulting in markedly reduced or absent β -globin chain synthesis. The imbalance between α - and β -globin chains leads to ineffective erythropoiesis, chronic hemolytic anemia, and progressive iron overload. In pediatric patients, the disease manifests early in life with profound anemia, hepatosplenomegaly, growth retardation, and skeletal deformities secondary to bone marrow expansion. Despite advances in transfusion protocols and iron chelation therapy, hemolysis remains a central contributor to morbidity (1,2).

Hemolysis in β -thalassemia major is multifactorial. Intrinsic defects, including oxidative membrane damage, altered cytoskeletal integrity, and phosphatidylserine externalization, render erythrocytes structurally fragile and immunologically recognizable. Extrinsic mechanisms, particularly splenic sequestration and immune-mediated clearance, further shorten red blood cell (RBC) lifespan. Among these immune mechanisms, activation of the complement system has emerged as an important but underappreciated contributor to erythrocyte destruction. Complement deposition on damaged RBC membranes enhances opsonization, promotes phagocytosis, and in certain contexts may lead to membrane attack complex-mediated lysis (3,4)..

The spleen plays a dual role in β -thalassemia major: it serves as the primary site for removal of abnormal erythrocytes and as a regulator of immune and complement-mediated responses. Hypersplenism commonly develops as a consequence of chronic hemolysis and extramedullary hematopoiesis, leading to increased transfusion requirements. Splenectomy is therefore often considered in selected pediatric patients to reduce transfusion burden and mitigate extravascular hemolysis. However, removal of the spleen alters immune homeostasis, including complement regulation and clearance of opsonized cells, potentially shifting the pattern of hemolysis from predominantly extravascular to intravascular mechanisms (5,6).

Erythrocytes are normally protected from autologous complement injury by membrane-bound regulatory proteins such as CD55 (decay-accelerating factor) and CD59 (membrane inhibitor of reactive lysis). In β -thalassemia major, oxidative stress and repeated transfusions may affect the expression or function of these complement regulators, increasing susceptibility to complement-mediated damage. Following splenectomy, impaired clearance of complement-coated erythrocytes and circulating immune complexes may further amplify complement activation, with implications for hemolysis, inflammation, and thromboembolic risk (7,8).

This review explores the interplay between splenic function, complement regulation on erythrocytes, and hemolytic mechanisms in pediatric β -thalassemia major. By integrating current insights into immunohematology and clinical outcomes. To clarify how splenectomy influences complement dynamics and to identify potential therapeutic strategies to optimize erythrocyte survival while minimizing complications.

2. Beta-Thalassemia Major and Splenectomy

Beta-thalassemia major (BTM) is caused by mutations that severely reduce β -globin synthesis, requiring patients to rely on blood transfusions, which increase iron overload risks. The spleen plays a critical role in preventing infections, and its absence raises susceptibility. Splenectomy may reduce immune complications but is avoided in children under 5 due to its immune significance. Hyposplenism and electrolyte imbalances might prolong red blood cell lifespan, but the effects of splenectomy on complement regulatory proteins in erythrocytes of BTM children remain unstudied (2,9).

Complement activation influences hemolysis, with erythrocytes potentially affecting beta-thalassemia hemolysis levels. The absence of peptide 28H798, mimicking CD59w's cytoplasmic tail, affects susceptibility to transfusion-related immunomodulation. Variations in complement regulatory protein levels are associated with immune disturbances in thalassemia patients. Hyposplenism or asplenia could change immune responses to protein-conjugated antigens in thalassemia and sickle cell disease, suggesting lower complement regulatory protein expression in erythrocytes of splenectomized children with beta-thalassemia major (9,10).

3. Complement Regulatory Proteins: Biology and Relevance in Erythrocytes

Essential complement regulator proteins include CD55/DAF, CD59, and CD46. Rh phenotypically express only CD55 and CD59. CD55, or decay accelerating factor (DAF), interrupts complement amplification by downregulating C3 and C5 convertase activity. CD59 inhibits MAC formation by binding C5b-8 and preventing C9 polymerisation. β -thalassemia major erythrocytes express decreased levels of CD55 and CD59. Additionally, complement components, such as C3 and C4, persist within normal ranges due to spleen function (11,12).

Erythrocytes contribute to complement regulatory processes. Erythrocyte surface DAF prevents complement attack through opsonisation, while CD59 restricts membrane attack complex formation. Inhibiting either function increases susceptibility to in vitro complement lysis. Erythrocyte CD46 expression facilitates decreased β -thalassemia major survival through conjugation with *Plasmodium falciparum*. The pattern of complement regulatory proteins displayed by other cellular compartments can be dominated by hemolysis. In spleen-intact subjects, more than a quarter of complement regulation takes place within the immune milieu. The implications of splenectomy on either erythrocyte surface expression level remain underexplored (12-14).

4. Rationale for Investigating Splenectomy Effects on Complement Regulation in Pediatric Beta-Thalassemia Major

Complement regulatory proteins on erythrocytes help maintain homeostasis and prevent complement-mediated damage. In beta-thalassemia major patients, complement activation occurs, yet the up-regulation of these proteins (CD55, CD59, CD46) after splenectomy is not well understood. Splenectomy may enhance hemolysis and increase pro-inflammatory cytokine release, altering erythrocyte enzymatic activity and potentially affecting immune and complement system interactions. However, the effects of splenectomy on complement regulatory protein expression in children and adolescents with beta-thalassemia major remain unexplored (2,5,15,16).

Increased hemolysis from reduced protein expression is the primary effect of splenectomy on erythrocytes in children and adolescents with beta-thalassemia major. Splenectomy may affect electrolyte balance and immune response, impairing defense against complement-mediated destruction and leading to late complications. Further longitudinal studies on erythrocyte integrity pre- and post-splenectomy are needed, along with more data on transfusion-related issues, as fewer transfusions might alter erythrocyte surface protein expression due to long-term iron overload (17,18).

Pediatric patients represent a distinct immunologic population in which long-term consequences of splenectomy extend into adulthood. Early surgical intervention may permanently reshape immune surveillance and complement homeostasis. Identifying biomarkers of complement activation and erythrocyte susceptibility could guide individualized decisions regarding timing of splenectomy, transfusion strategies, and potential complement-modulating therapies (19,20).

Collectively, exploring the relationship between splenectomy and complement regulation in pediatric β -thalassemia major addresses a critical gap in understanding the immunopathophysiology of hemolysis. Such insights may support more targeted therapeutic approaches aimed at preserving erythrocyte survival while minimizing inflammatory and thrombotic complications (21).

5. Clinical Implications

The results shed light on how splenectomy alters complement regulator concentration in the erythrocytes of children and adolescents with β -thalassemia major. Knowledge of these changes may help tailor transfusion strategies and estimate the consequences of splenectomy on complement-mediated immunological responses and erythrocyte survival (17,22).

Erythrocyte-recognized haemolytic disorders and the spleen's role are essential for transfusions in non-splenectomized patients. Preventing splenectomy is crucial, but careful evaluation of surgery is important, especially in children and adolescents with beta-thalassemia major. Frequent transfusions lead to high iron overload, necessitating assessments due to risks like chronic immunosuppression and infections. Early splenectomy can prevent severe issues in patients not frequently transfused with hypotransferrinaemia (16,19,22,23).

Understanding the interaction between splenectomy and complement regulation in pediatric β -thalassemia major has important implications for patient management, risk stratification, and therapeutic innovation (22):

Optimization of Splenectomy Timing and Patient Selection:

Clarifying how splenectomy alters complement-mediated hemolysis may refine criteria for surgical intervention. While splenectomy can reduce transfusion requirements and alleviate hypersplenism, it may simultaneously predispose patients to persistent complement activation and intravascular hemolysis. Identifying biomarkers such as complement deposition on erythrocytes, reduced CD55/CD59 expression, or elevated soluble complement components could help determine which patients are most likely to benefit from surgery and which may be at higher risk of post-splenectomy complications (24,25).

Monitoring Hemolysis and Complement Activation:

Routine hematologic indices (reticulocyte count, LDH, bilirubin) provide indirect evidence of hemolysis but do not distinguish between extravascular and intravascular mechanisms. Incorporating assays that assess complement activity (e.g., C3/C4 levels, CH50, complement split products) may offer more precise evaluation of hemolytic pathways, particularly in splenectomized patients with unexplained anemia or increasing transfusion needs (20,23).

Thromboembolic Risk Assessment

Post-splenectomy β -thalassemia major patients exhibit an increased risk of thrombosis and pulmonary hypertension. Complement activation contributes to endothelial injury, platelet activation, and a pro-inflammatory, pro-coagulant milieu. Enhanced surveillance for thrombotic events, along with consideration of antiplatelet or anticoagulant prophylaxis in high-risk individuals, may be warranted. Understanding complement-mediated mechanisms may also guide targeted preventive strategies (22,24).

Implications for Transfusion Practices

Chronic transfusion therapy introduces donor erythrocytes that may vary in complement regulatory protein expression and susceptibility to immune-mediated injury. In splenectomized patients, altered immune clearance may prolong the circulation of damaged or complement-coated cells. Tailored transfusion protocols, extended phenotypic matching, and monitoring for alloimmunization may help reduce immune activation and complement amplification (25).

Potential for Complement-Targeted Therapies

Emerging complement inhibitors used in other hemolytic disorders raise the possibility of therapeutic modulation in selected cases of β -thalassemia major with significant complement-mediated hemolysis. Although not yet standard of care, targeted inhibition of complement pathways could represent a future adjunctive strategy, particularly in patients demonstrating persistent intravascular hemolysis after splenectomy (21,26).

Long-Term Immune and Infection Considerations

Beyond hemolysis, splenectomy affects immune surveillance and increases susceptibility to encapsulated bacterial infections. Complement plays a crucial role in opsonization and bacterial clearance. Thus, strict adherence to vaccination schedules, prophylactic antibiotics in selected pediatric patients, and long-term infection monitoring remain essential components of care (27,28).

6. Conclusion

Splenectomy in beta-thalassemia major impacts complement regulatory proteins, making patients more susceptible to complement damage. The downregulation of CD59 on erythrocytes suggests reduced defense against complement activity, indicating weaker responses to hemolytic stress.

The incorporation of complement regulation into the management of pediatric β -thalassemia enhances decision-making processes, diminishes hemolysis, and informs therapeutic approaches.

Additionally, lower CD55 levels in a high-transfusion, immune-suppressive setting pose risks of transfusion-related infections post-splenectomy. Reduced CA levels, vital for controlling osmotic fragility and hemolysis, were also observed in splenectomized patients, highlighting the need for further research on complement regulatory proteins and osmotic fragility in beta-thalassemia.

Overall, integrating immunologic and hematologic perspectives is critical to optimizing long-term outcomes in pediatric β -thalassemia major. Continued research into complement dynamics may help refine therapeutic approaches that preserve erythrocyte survival while minimizing systemic complications.

Long-term studies are needed to examine the effects of splenectomy on complement regulatory proteins like CD59 and their connections to the complement system and osmotic fragility, assisting physicians in informed decision-making.

Conflict of interest: The authors declare no conflict of interest.

Author contribution: Authors contributed equally in the study.

REFERENCES

1. Merchant, R. H., Shah, A. R., Ahmad, J., Karnik, A., & Rai, N. (2015). Post splenectomy outcome in β -thalassemia. *The Indian Journal of Pediatrics*, 82(12), 1097-1100.
2. Kurtogllu, A. U., Koçtekin, B., Kurtoglu, E., & Yildiz, M. (2019). The effect of splenectomy on complement regulatory proteins in erythrocytes in β -thalassemia major. *Archives of Medical Science*, 15(1), 191-195.
3. Atichartakarn, V., Chuncharunee, S., Archararit, N., Udomsubpayakul, U., & Aryurachai, K. (2014). Intravascular hemolysis, vascular endothelial cell activation and thrombophilia in splenectomized patients with hemoglobin E/ β -thalassemia disease. *Acta Haematologica*, 132(1), 100-107.
4. Ali, S., Mumtaz, S., Shakir, H. A., Khan, M., Tahir, H. M., Mumtaz, S., ... & Khan, M. A. (2021). Current status of beta-thalassemia and its treatment strategies. *Molecular genetics & genomic medicine*, 9(12), e1788.
5. Ammar, S. A., Elsayh, K. I., Zahran, A. M., & Embaby, M. (2014). Splenectomy for patients with β -thalassemia major: long-term outcomes. *The Egyptian Journal of Surgery*, 33(4).
6. Gluba-Brzózka, A., Franczyk, B., Rysz-Górczyńska, M., Rokicki, R., Koziarska-Rościszewska, M., & Rysz, J. (2021). Pathomechanisms of immunological disturbances in β -thalassemia. *International Journal of Molecular Sciences*, 22(18), 9677.
7. Obaid, J. M., Abo El-Nazar, S. Y., Ghanem, A. M., El-Hadidi, A. S., & Mersal, B. H. (2015). Compensation of CD55 underexpression on red blood cells of β -thalassemia major patients. *Hemoglobin*, 39(3), 184-189.
8. Evangelidis, P., Evangelidis, N., Vlachaki, E., & Gavrilaki, E. (2024). What is the role of complement in bystander hemolysis? Old concept, new insights. *Expert Review of Hematology*, 17(4-5), 107-116.
9. Zahira, A. F., Wasono, A. R. P., Yudho, S. U., & Bintoro, H. K. (2022). The Role of Splenectomy in The Treatment of β -Thalassemia Major Patients: A Literature Review. *The Role of Splenectomy in The Treatment of β -Thalassemia Major Patients: A literature review*, 115(1), 6-6.
10. Roumenina, L. T., Bartolucci, P., & Pirenne, F. (2019). The role of complement in post-transfusion hemolysis and hyperhemolysis reaction. *Transfusion medicine reviews*, 33(4), 225-230.
11. Maciel, T. T., Sbeih, N., Rignault-Bricard, R., Chevalme, I., Allali, S., Brochier, C., ... & Hermine, O. (2024). Complement Activation and Its Implication in the Pathophysiology of Hemolytic Anemia and Aging in Mouse Models of Sick Cell Disease and Beta-Thalassemia. *Blood*, 144, 3870.
12. Cho, H. (2015). Complement regulation: physiology and disease relevance. *Korean journal of pediatrics*, 58(7), 239.
13. Thielen, A. J., Zeerleder, S., & Wouters, D. (2018). Consequences of dysregulated complement regulators on red blood cells. *Blood Reviews*, 32(4), 280-288.
14. Alegretti, A. P., Schneider, L., Piccoli, A. K., & Xavier, R. M. (2012). The role of complement regulatory proteins

in peripheral blood cells of patients with systemic lupus erythematosus. *Cellular immunology*, 277(1-2), 1-7..

15. Darzi, A. A., Kamali, S., & Khakzad, M. (2015). Influence of splenectomy on immunoglobulins and complement components in major thalassemia. *Caspian journal of internal medicine*, 6(1), 30.
16. Awad, A. S., Ali, H. A., & Althalmi, S. A. A. (2023). Assessment of serum fractalkine level as potential role for complications in splenectomized and non-splenectomized patients with β -thalassemia major. *Anaesthesia, Pain & Intensive Care*, 27(5), 495-500.
17. Miri-Aliabad, G., Rezaeifar, A., & Salarzaei, M. (2022). Comparison of immunoglobulins status in splenectomized and non-splenectomized patients with major beta-thalassemia. *Journal of Pediatrics Review*, 10(2), 161-166.
18. Mizher, E. A. (2024). Immunological Changes and Complement Proteins in Major Thalassemia Patients Proteins Post-Splenectomy: Perubahan Immunologi dan Protein Komplemen pada Pasien Talasemia Mayor Protein Pasca-Splenektomi. *Academia Open*, 9(2).
19. Mahmoud, S. S., Mohamed, G. B., Hakeem, G. L. A., Higazi, A. M., Nafady, A. A. H., Farag, N. M., ... & Shaban, A. (2017). Comparison of the immunity status in-between children with [beta]-thalassaemia major receiving different treatment modalities: a single Egyptian district study. *Immunome Research*, 13(1), 1.
20. Beshir, M. R., Abd-Alaziz, M. B., Ismail, W. I., & Baz, E. G. (2024). Evaluation of Immune System Alterations in Children with β -Thalassemia Major: Single-Center Egyptian Study. *Zagazig University Medical Journal*, 30(6), 2280-2289.
21. Zahran, A. M., Shaltout, A. S., Hamada, I. H., Mohammed, A. M., Abo Elgheet, A. M., & Elsayh, K. I. (2020). Assessment of complement regulatory proteins CD55 and CD59 on erythrocytes in beta-thalassemia major patients. *Egypt J Immunol*, 27, 109-118.
22. McCarthy, P., & Smith, O. P. (2023). Hematological problems in pediatric surgery. In *Pediatric Surgery: Diagnosis and Management* (pp. 119-144). Cham: Springer International Publishing.
23. Sari, T. T., Gatot, D., Akib, A. A., Bardosono, S., Hadinegoro, S. R., Harahap, A. R., & Idjradinata, P. S. (2014). Immune response of thalassemia major patients in Indonesia with and without splenectomy. *Acta Medica Indonesiana*, 46(3).
24. Abo-Elwafa, H. A., Youseff, L. M., Mahmoud, R. A., Elbadry, M. I., Tawfeek, A., & Aziz, S. P. (2023). Venous thromboembolism risk assessment among Beta-thalassemia patients. *Journal of Applied Hematology*, 14(3), 230-235.
25. Lal, A., Wong, T. E., Andrews, J., Balasa, V. V., Chung, J. H., Forester, C. M., ... & Vichinsky, E. P. (2018). Transfusion practices and complications in thalassemia. *Transfusion*, 58(12), 2826-2835.
26. Bazi, A., Shahramian, I., Yaghoobi, H., Naderi, M., & Azizi, H. (2018). The role of immune system in thalassemia major: a narrative review. *Journal of Pediatrics Review*, 6(2), 29-36.
27. Zanetti, R. C., Vasta, L. M., Romanelli, K., & Newton, T. C. (2021). Management of hyperhemolysis in β -thalassemia with multiple immunosuppressives, including complement blockade. *Journal of pediatric hematology/oncology*, 43(8), e1145-e1147.
28. Caocci, G., Mulas, O., Barella, S., Orecchia, V., Mola, B., Costa, A., ... & La Nasa, G. (2023). Long-term health-related quality of life and clinical outcomes in patients with β -thalassemia after splenectomy. *Journal of clinical medicine*, 12(7), 2547.

..