Thalassemia as a Hypercoagulable State

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Abstract

Although the life expectancy of thalassemia patients has markedly improved over the last few decades, patients still suffer from many complications of this congenital disease. The presence of a high incidence of thromboembolic events, mainly in thalassemia intermedia, has led to the identification of a hypercoagulable state in these patients. In this article, the molecular and cellular mechanisms leading to hypercoagulability in thalassemia are highlighted, with a special focus on thalassemia intermedia, being the type with the highest incidence of thrombotic events as compared with other types of thalassemia. Clinical experience and available clues regarding optimal management are also discussed.

Keywords

Thalassemia, hypercoagulability, thromboembolism, stroke, splenectomy, transfusion

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Phenotypic diversity within the β-thalassemia syndromes has traditionally received considerable interest, with several molecular and environmental modifiers of disease severity so far described.[1] Patients with transfusion-dependent β-thalassemia major (TM) suffer the most severe form and show the highest mortality rates.[1] Nevertheless, the introduction of safe transfusion practices and effective iron chelation therapy continues to improve patient survival, allowing for several clinical complications to have time to manifest.[2] The term β-thalassemia intermedia (TI) was first suggested to describe patients who have milder anemia compared with patients with TM and who usually present to medical attention later in childhood and remain largely transfusion-independent. However, recent evidence suggests that the diagnosis of TI carries higher morbidity than previously recognized, especially in the transfusion-independent patient, where the mechanism of disease remains largely unbalanced.[3]

Three main factors highlight the pathophysiology of TI: ineffective erythropoiesis, chronic anemia/hemolysis, and iron overload secondary to increased intestinal absorption.[3] The extreme diversity in phenotypic expression within the diagnosis of TI itself led to a wide variation in observed clinical complications and management practices.[3,4] Among the medical complications of TI that were found to occur at high rates, even more frequently than in patients with TM, are thromboembolic events (TEE).[5] Here, we review current evidence on TEE in thalassemia patients, with special emphasis on TI.

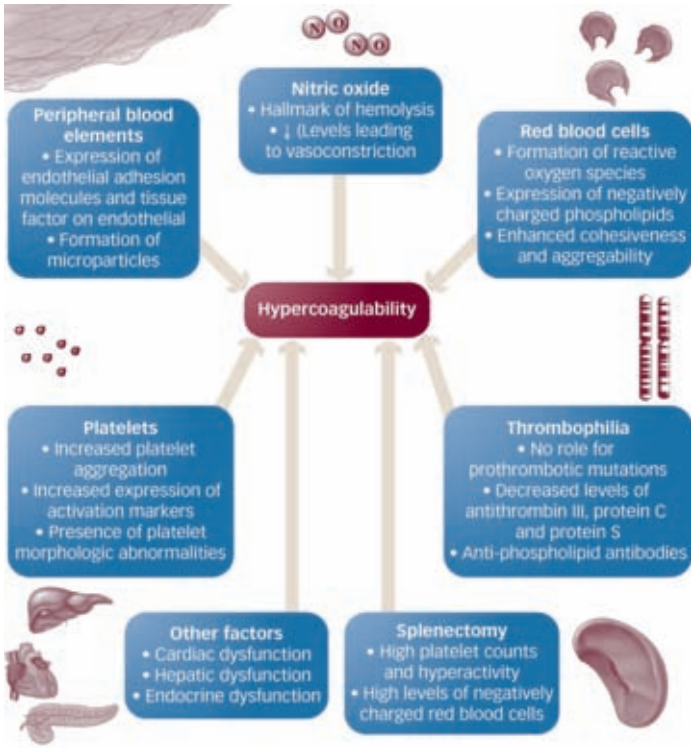
Pathophysiology

Hypercoagulability in patients with thalassemia has been attributed to several factors (see *Figure 1*).[5,6] It is often a combination of these factors that leads to TEE.

It is widely accepted that patients with thalassemia have chronically activated platelets and enhanced platelet aggregation,[7] as confirmed by the increased expression of CD62P (P-selectin) and CD63, markers of *in vivo* platelet activation.[8,9] Splenectomised thalassemia patients have high platelet counts,[10,11] but with a shorter lifespan due to enhanced consumption.[12] It has also been shown that splenectomized TM and non-splenectomized TI patients have four to 10 times higher levels of metabolites of prostacyclin (PGI2) and thromboxane A2, both markers of haemostatic activity, than controls. However, no significant difference was found between TM and TI patients.[13]

Furthermore, the oxidation of globin subunits in thalassemia erythroid cells leads to the formation of hemichromes[14] which precipitate, instigating hem disintegration and the eventual release of toxic non-transferrin-bound iron species.[15] The free iron in turn catalyzes the formation of reactive oxygen species, leading to oxidation of membrane proteins and formation of red-cell 'senescence' antigens such as phosphatidylserine,[16] which cause the thalassaemic red blood cells (RBCs) to become rigid and deformed and to aggregate, resulting in premature cell removal.[17] Thalassemic RBCs with negatively charged

Figure 1: Factors Contributing to Hypercoagulability in Thalassemia



Circles represent adjusted odds ratios and whiskers represent 95 % confidence intervals. Source: Reproduced from Taher et al., 2010[4] and Cappellini et al., 2010.[5]

phospholipids increase thrombin generation,[18,19] as evidenced by studies using annexin V, a protein with high affinity and specificity for anionic phospholipids.[19] Splenectomized patients have a substantially higher number of these negatively charged RBCs and in turn show higher thrombin generation.[20,21] TI patients were also found to have higher levels of procoagulant microparticles of RBC, leukocytic and endothelial origins compared with controls;[22] the contribution of these fragments to thromboembolic events in TI is under investigation.

The presence of other peripheral blood elements in thalassemia patients, such as E-selectin (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), von Willebrand factor (VWF) and vascular cell adhesion molecule-1 (VCAM-1), indicates that endothelial injury or activation may be an aspect of the disease, aiding in the recruitment of white blood cells and RBCs and promoting thrombosis.[23,24] Studies have demonstrated that RBCs from TM and TI patients show increased adhesion to cultured endothelial cells (EC).[25]

Inherited thrombophilia does not play a role in the hypercoagulability of thalassemia,[26,27] but high levels of antiphospholipid antibodies and low protein C and S levels have been documented.[6] The presence of cardiac, hepatic or endocrine dysfunction in patients with severe iron overload may also contribute to hypercoagulability in thalassemia.[6]

Clinical Implications

Epidemiologic data on TEE in thalassemia are scarce. Borgna-Pignatti et al. surveyed nine Italian pediatric thalassemia centers, observing that 4 % of the 683 patients with TM and 9.6 % of the 52 patients with TI had experienced a TEE.[28] The same group showed six years later that 1.1 % of 720 patients with TM in seven Italian centres had thrombosis.[2] Cappellini et al. followed up 83 patients with TI over 10 years, 82 of whom were splenectomized and found that 29 % (24/83) experienced a venous TEE.[21] One study directly implicated TEE as the cause of death in 2.5 % of transfusion-dependent thalassemia patients.[29] After examining data from 8,860 patients in the Mediterranean area and Iran, Taher et al. observed that TEE occurred 4.38 times (95 % confidence interval [CI] 3.14–6.10, p<0.001) more frequently in TI than TM, with more venous events occurring in TI and more arterial events occurring in TM.[30] It was found that 14 % of mortalities in the whole group were due to TEE. Age above 20 years, splenectomy, family history of TEE and previous TEE were identified as the main risk factors for thrombosis in TI. Furthermore, the study showed that 68 % of TI patients that had a TEE had an average hemoglobin level of <9 g/dl and only 33 % were receiving regular blood transfusions, whereas 94 % were splenectomized. Moreover, patients receiving aspirin therapy had a significantly lower rate of recurrent TEE.[30]

The evidence for brain involvement in thalassemia dates back to 1972, when 20 % of 138 TM patients in Greece were found to have neurologic deficits compatible with transient ischemic attacks (TIAs).[31] Further evidence of TIAs causing neurological symptoms, such as headaches, hemiparesis and seizures, was shown in 2.2 % of patients with TM in Italy.[28] Although overt stroke occurs more frequently in TM than TI (28 versus 9 %, respectively),[30] it has been shown that as many as 37.5 % of patients with TI have asymptomatic brain damage on magnetic resonance imaging (MRI).[31] A more recent study on Lebanese patients determined that splenectomized adults with TI show a rate of silent white matter lesions as high as 60 %.[32] The occurrence and multiplicity of the lesions were associated with older age (mean age of 36.1 years for lesion-positive patients versus 26.1 years for lesion-negative patients) and transfusion naivety (83.3 % of lesion-positive patients have never had a transfusion versus 25 % of lesion-negative patients).[32] Another study from Iran followed to confirm these findings.[33]

In order to obtain much-needed clinical data concerning the optimal management of patients with TI, the Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity (OPTIMAL CARE) study evaluated 584 patients with TI at six comprehensive care centres (Lebanon, Italy, Iran, Egypt, United Arab Emirates and Oman) for the associations between patient and disease characteristics, treatment received and the rate of clinical complications.[4] Thrombosis was the fifth most common complication, affecting 14 % of the patient population. On multivariate analysis, splenectomy, age above 35 years and a serum ferritin level ≥1,000 µg/l were associated with a higher risk of thrombosis.[4] Conversely, a positive history of transfusion and a hemoglobin level ≥9 g/dl were found to be protective against thrombosis (see *Table 1*).[4] A higher occurrence of TEE with advancing age was also observed.[34] In an effort to further understand the effect of splenectomy on the risk of TEE, a substudy of OPTIMAL CARE examined the characteristics of splenectomized patients with TI who developed TEE, aiming to identify high-risk patients who deserve further consideration for preventive strategies.[35] Splenectomized patients with documented TEE (Group I, n=73) were age- and sex-matched to splenectomized patients without TEE (Group II) and non-splenectomized patients without TEE (Group III). The

study determined that splenectomized TI patients who experience TEE are characterized by high nucleated RBC (≥300 x 106/l) and platelet counts (≥500 x 109/l) and are more likely to have evidence of pulmonary hypertension (PHT) and be transfusion-naive. As such, it was suggested that splenectomized TI patients at risk of developing TEE may be identified early on by these laboratory markers, presence of PHT and transfusion status.[35] The study further examined how long it took for a TEE to develop following splenectomy and found the median time to thrombosis to be eight years.[35] This delay indicates that TEE in splenectomized patients with TI is not an acute complication, but a manifestation of a chronic underlying process, further emphasizing the need for a long-term treatment modality for prevention.[35]

Potential Preventive Strategies

Reduction of the proportion of circulating RBCs with thrombogenic potential may be achieved by introducing blood transfusions and may account for the lower rate of TEE in transfused versus non-transfused patients in previous studies.[4,30,32,35] As such, transfusion therapy may be worthwhile to prevent the occurrence of TEE and other complications[3] in TI patients for whom current practice does not necessarily recommend transfusions. Rather than enforcing the regular transfusion regimens implemented in TM, blood transfusion, if initiated in patients with TI, should be individually tailored to meet patient needs. Although introduction of blood transfusions will increase the rate of iron accumulation, effective methods of iron chelation are now available and the benefits of transfusion therapy may greatly outweigh the cost and inconvenience of iron chelation therapy.[36] This approach requires prospective evaluation. Since splenectomy is a major contributor to TEE in patients with thalassemia,[37] reassessment of the procedure and appropriate risk–benefit evaluation prior to any attempt at splenectomy are called for. This is also essential in line with recent evidence on the high rates of other clinical complications after splenectomy,[4] alongside the well-known increased susceptibility to infection.[38]

The literature lacks proper evidence on the role of antiplatelet or anticoagulant agents in the management of thalassemia.[6] The lower recurrence rate of TEE in TI patients who took aspirin after their first TEE, when compared with those who did not, suggests a potential role for aspirin.[30] Moreover, the association of higher platelet counts with TEE in patients with TI further suggests a role for aspirin in this patient population.[35] Fetal hemoglobin-inducing agents, such as hydroxycarbamide decitabine and decitabine, were also shown to lower plasma markers of thrombin generation.[5] Hydroxycarbamide may modulate hypercoagulability in several ways: it may reduce phospholipid expression on the surface of RBCs and platelets and decrease RBC adhesion to thrombospondin, a thrombin-sensitive protein.[5] It may also decrease the count of leukocytes, particularly monocytes expressing transcription factor, in addition to being a nitric oxide donor.[39]

Table 1: Predictors of Thrombosis in the Overview on Practices in Thalassemia Intermedia Management Aiming for Lowering Complication Rates Across a Region of Endemicity Study

Parameter	Adjusted OR	95 % CI	p-value
Age >35	2.59	1.39–4.87	0.003
Female	1.27	0.74–2.19	0.387
Hemoglobin ≥9 g/dl	0.41	0.23–0.71	0.001
Ferritin ≥1,000 µg/l	1.86	1.09–3.16	0.023
Splenectomy	6.59	3.09–14.05	<0.001
Transfusion	0.28	0.16–0.48	<0.001
Hydroxycarbamide	0.56	0.28–1.10	0.090
Iron chelation	0.97	0.56–1.68	0.912

CI = confidence interval; OR = odds ratio.

It is recommended that each patient be assessed individually and assigned a personalized thrombotic risk based on intrinsic and extrinsic factors. High nucleated RBC and platelet counts, evidence of PHT and transfusion naivety can be used as indicators of TEE for splenectomised patients with TI and could be practical in the clinical setting.[35] Such a risk assessment model would be valuable in identifying high-risk patients and targeting them for further testing. Several diagnostic tests are being explored to help identify patients at risk, with promising preliminary results.[40]

The hypercoagulable state in thalassemia is due to multiple elements, a combination of which is often the drive behind a clinical TEE. Splenectomy and transfusion naivety are increasingly highlighted as important risk factors for TEE, especially in patients with TI. An individualized approach is recommended to establish an optimal strategy for preventing the occurrence of this complication of thalassemia. ■

1. Steinberg MH, Forget BG, Higgs DR, Weatherall DJ, *Disorders of hemoglobin: genetics, pathophysiology, and clinical management, 2nd edn*, Cambridge: Cambridge University Press, 2009.
2. Borgna-Pignatti C, Rugolotto S, De Stefano P, et al., Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine, *Haematologica*, 2004;89:1187–93.
3. Taher AT, Musallam KM, Cappellini MD, Weatherall DJ, Optimal management of beta thalassemia intermedia, *Br J Haematol*, 2011;152:512–23.
4. Taher AT, Musallam KM, Karimi M, et al., Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study, *Blood*, 2010;115:1886–92.
5. Cappellini MD, Motta I, Musallam KM, Taher AT, Redefining thalassemia as a hypercoagulable state, *Ann N Y Acad Sci*, 2010;1202:231–6.
6. Taher AT, Otrock ZK, Uthman I, Cappellini MD, Thalassemia and hypercoagulability, *Blood Rev*, 2008;22:283–92.
7. Winichagoon P, Fucharoen S, Wasi P, Increased circulating platelet aggregates in thalassemia, *Southeast Asian J Trop Med Public Health*, 1981;12:556–60.
8. Del Principe D, Menichelli A, Di Giulio S, et al., PADGEM/GMP-140 expression on platelet membranes from homozygous beta thalassaemic patients, *Br J Haematol*, 1993;84:111–7.
9. Ruf A, Pick M, Deutsch V, et al., *In-vivo* platelet activation correlates with red cell anionic phospholipid exposure in patients with beta-thalassemia major, *Br J Haematol*, 1997;98:51–6.
10. Cappellini MD, Grespi E, Cassinerio E, et al., Coagulation and splenectomy: an overview, *Ann N Y Acad Sci*, 2005;1054:317–24.
11. Atichartakarn V, Angchaisuksiri P, Aryurachai K, et al., *In vivo* platelet activation and hyperaggregation in hemoglobin E/beta-thalassemia: a consequence of splenectomy, *Int J Hematol*, 2003;77:299–303.
12. Eldor A, Krausz Y, Atlan H, et al., Platelet survival in patients with beta-thalassemia, *Am J Hematol*, 1989;32:94–9.
13. Eldor A, Lellouche F, Goldfarb A, et al., *In vivo* platelet activation in beta-thalassemia major reflected by increased platelet-thromboxane urinary metabolites, *Blood*, 1991;77:1749–53.
14. Rund D, Rachmilewitz E, Beta-thalassemia, *N Engl J Med*, 2005;353:1135–46.
15. Hershko C, Graham G, Bates GW, Rachmilewitz EA, Non-specific serum iron in thalassemia: an abnormal serum iron fraction of potential toxicity, *Br J Haematol*, 1978;40:255–63.
16. Kuypers FA, de Jong K, The role of phosphatidylserine in recognition and removal of erythrocytes, *Cell Mol Biol (Noisy-le-grand)*, 2004;50:147–58.
17. Tavazzi D, Duca L, Graziadei G, et al., Membrane-bound iron contributes to oxidative damage of beta-thalassemia intermedia erythrocytes, *Br J Haematol*, 2001;112:48–50.
18. Borenstain-Ben Yashar V, Barenholz Y, Hy-Am E, et al., Phosphatidylserine in the outer leaflet of red blood cells from beta-thalassemia patients may explain the chronic hypercoagulable state and thrombotic episodes, *Am J Hematol*, 1993;44:63–5.
19. Helley D, Eldor A, Girot R, et al., Increased procoagulant activity of red blood cells from patients with homozygous sickle cell disease and beta-thalassemia, *Thromb Haemost*, 1996;76:322–7.
20. 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;118:893–8.
21. Cappellini MD, Robbiolo L, Bottasso BM, et al., Venous thromboembolism and hypercoagulability in splenectomized patients with thalassemia intermedia, *Br J Haematol*, 2000;111:467–73.
22. Habib A, Kunzelmann C, Shamseddeen W, et al., Elevated levels of circulating procoagulant microparticles in patients with beta-thalassemia intermedia, *Haematologica*, 2008;93:941–2.
23. Butthep P, Bunyaratvej A, Funahara Y, et al., Alterations in vascular endothelial cell-related plasma proteins in

thalassaemic patients and their correlation with clinical symptoms, *Thromb Haemost*, 1995;74:1045–9.
24. Butthep P, Bunyaratvej A, Funahara Y, et al., Possible evidence of endothelial cell activation and disturbance in thalassemia: an in vitro study, *Southeast Asian J Trop Med Public Health*, 1997;28(Suppl. 3):141–8A.
25. Hovav T, Goldfarb A, Artmann G, et al., Enhanced adherence of beta-thalassaemic erythrocytes to endothelial cells, *Br J Haematol*, 1999;106:178–81.
26. Zalloua PA, Shbaklo H, Mourad YA, et al., Incidence of thromboembolic events in Lebanese thalassemia intermedia patients, *Thromb Haemost*, 2003;89:767–8.
27. Iolascon A, Giordano P, Storelli S, et al., Thrombophilia in thalassemia major patients: analysis of genetic predisposing factors, *Haematologica*, 2001;86:1112–3.
28. Borgna-Pignatti C, Carnelli V, Caruso V, et al., Thromboembolic events in beta thalassemia major: an Italian multicenter study, *Acta Haematol*, 1998;99:76–9.
29. Zurlo MG, De Stefano P, Borgna-Pignatti C, et al., Survival and causes of death in thalassemia major, *Lancet*, 1989;2:27–30.
30. Taher A, Isma'eel H, Mehio G, et al., Prevalence of thromboembolic events among 8,860 patients with thalassemia major and intermedia in the Mediterranean area and Iran, *Thromb Haemost*, 2006;96:488–91.
31. Manfre L, Giarratano E, Maggio A, et al., MR imaging of the brain: findings in asymptomatic patients with thalassemia intermedia and sickle cell-thalassemia disease, *AJR Am J Roentgenol*, 1999;173:1477–80.
32. Taher AT, Musallam KM, Nasreddine W, et al., Asymptomatic brain magnetic resonance imaging abnormalities in splenectomized adults with thalassemia intermedia, *J Thromb Haemost*, 2010;8:54–9.
33. Karimi M, Bagheri H, Rastgu F, Rachmilewitz EA, Magnetic resonance imaging to determine the incidence of brain ischaemia in patients with beta-thalassemia intermedia, *Thromb Haemost*, 2010;103:989–93.
34. Taher AT, Musallam KM, El-Beshlawy A, et al., Age-related complications in treatment-naive patients with thalassemia intermedia, *Br J Haematol*, 2010;150:486–9.
35. Taher AT, Musallam KM, Karimi M, et al., Splenectomy and thrombosis: the case of thalassemia intermedia, *J Thromb Haemost*, 2010;8:2152–8.
36. Taher A, Hershko C, Cappellini MD, Iron overload in thalassemia intermedia: reassessment of iron chelation strategies, *Br J Haematol*, 2009;147:634–40.
37. Crary SE, Buchanan GR, Vascular complications after splenectomy for hematologic disorders, *Blood*, 2009;114:2861–8.
38. Cadili A, de Gara C, Complications of splenectomy, *Am J Med*, 2008;121:371–5.
39. Sumiyoshi A, Thakerngpol K, Sonakul D, Pulmonary microthromboemboli in thalassemic cases, *Southeast Asian J Trop Med Public Health*, 1992;23(Suppl. 2):29–31.
40. Tripodi A, Cappellini MD, Chantarangkul V, et al., Hypercoagulability in splenectomized thalassemic patients detected by whole-blood thromboelastometry, but not by thrombin generation in platelet-poor plasma, *Haematologica*, 2009;94:1520–7.