Severe Transplant-Associated Thrombotic microangiopathy in Patients with Hemoglobinopathies

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Key words: Hemoglobinopathies, transplant-associated thrombotic microangiopathy, eculizumab

Short running title: TA-TMA in patients with hemoglobinopathies

Total word count: Abstract: 95**, Main text:**1,200

Total number of Tables: 1, Total number of figures: 1

Abbreviation list

TA-TMA	Transplant Associated Thrombotic Microangiopathy
AKI	Acute Kidney Injury
CKD	Chronic Kidney Disease
HCT	Hematopoietic Cell transplant
SCD	Sickle Cell Disease
HUS	Hemolytic Uremic Syndrome
TTP	Thrombotic Thrombocytopenic purpura
HTN	Hypertension
MAC	Myeloablative conditioning
GvHD	Graft Versus Host Disease
PEF	Pericardial effusion
CRRT	Continuous renal replacement therapy
PRES	Posterior Reversible Encephalopathy Syndrome
Bu/Cy/rATG	Busulfan, cyclophosphamide and rabbit anti-thymocyte globulin
RSV	Respiratory Syncytial virus
CECs	Circulating endothelial cells
ICAM-1	Intracellular adhesion molecule 1
VCAM-1	Vascular cell adhesion molecule 1

eDAMPs	Erythrocyte damage associated molecular patterns
eGFR	Estimated Glomerular filtration rate
LDH	Lactate dehydrogenase
CAE units	Complement Activity enzyme units
CFH	Complement factor H

Abstract

Incidence and severity of transplant-associated thrombotic microangiopathy (TA-TMA) in patients with hemoglobinopathies receiving hematopoietic cell transplant is unknown. We report the outcomes for two patients with TA-TMA who received eculizumab. A 2.5 year old male with sickle cell disease developed TA-TMA-associated pericardial tamponade, severe hypertension and acute kidney injury two months post-transplant. A seven-year-old female with beta-thalassemia major developed TA-TMA-related AKI, severe hypertension and seizures at six months post-transplant. Both patients progressed to chronic kidney disease (CKD). In patients with hemoglobinopathies, preexisting endothelial dysfunction may place them at greater risk for TA-TMA and subsequent CKD.

Introduction

Hematopoietic cell transplant (HCT) is a curative option for patients with hemoglobinopathies like thalassemia and sickle cell disease (SCD). These patients have endothelial dysfunction as a result of ongoing hemolysis [1-4]. It is unknown whether this pre-existing pathology places these patients at higher risk of transplant associated thrombotic microangiopathy (TA-TMA), a condition associated with widespread vascular injury. Incidence and severity of TA-TMA in this population undergoing HCT is unknown.

TA-TMA belongs to the family of thrombotic microangiopathies including hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). Endothelial injury leads to thrombosis, fibrin deposition, platelet consumption, and microangiopathic hemolytic anemia [5]. Clinical manifestations depend on the predominant organ microcirculation affected. Patients may present with intractable hypertension (HTN), acute kidney injury (AKI), pulmonary hypertension, polyserositis or multi-organ failure. TA-TMA is associated with considerable morbidity and mortality; as affected patients are 4.3 times more likely to develop chronic kidney disease (CKD) and have higher non-relapse mortality rate (43.6% vs 7.8%) than unaffected patients [6]. We describe two children with hemoglobinopathies who developed severe clinical manifestations of TA-TMA post HCT, despite receiving eculizumab therapy

Case Reports

Case 1

A 2.5 year old African male with severe SCD underwent single-unit, sibling cord blood transplant following myeloablative conditioning (MAC) with busulfan, cyclophosphamide and rabbit anti-thymocyte globulin (Bu/Cy/rATG). Tacrolimus and mycophenolate mofetil were given for graft-versus-host disease (GvHD) prophylaxis. Sixty-eight days post-transplant he presented with acute respiratory distress secondary to pericardial effusion (PEF) with tamponade. He was normotensive with normal blood counts and therapeutic tacrolimus levels. He underwent pericardiocentesis, which did not identify an infectious cause and therefore treatment with high-dose methylprednisone was started. After 1 week of therapy he developed recurrent PEF necessitating a pericardial drain, progressive pancytopenia, refractory hypertension (requiring eight anti-hypertensives) and AKI requiring continuous renal replacement therapy (CRRT). Laboratory testing was consistent with TA-TMA (Table I). Despite discontinuing tacrolimus, his symptoms worsened. Therefore, one week later eculizumab therapy was initiated. Dosing schedule was as follows: 1200 mg/week (week 1), 900 mg/week (weeks 2-3), 600 mg/week (weeks 4-8), 300 mg/week (weeks 9-17) and 300 mg/2 weeks (weeks 18-21). Hematological abnormalities normalized and effusion resolved after 4 weeks of therapy. CRRT was discontinued after 2 weeks and nephrotic range proteinuria resolved after 21 weeks. However, he progressed to CKD with HTN (requiring two anti-HTN medications). Currently, he is 30 months post-transplant and has an estimated GFR (eGFR) of 45 ml/min/1.73m^2 (pre-transplant eGFR 128ml/min/1.73m^2).

Case 2

A seven-year-old Asian female with β-thalassemia major on chronic transfusion therapy, underwent a 10/10 matched unrelated donor bone marrow transplant following MAC with fludarabine, Bu/Cy/rATG and GvHD prophylaxis with cyclosporine and methotrexate. Post-transplant course was complicated by grade II acute GvHD, posterior reversible encephalopathy syndrome (PRES) with intracranial bleed, gastrointestinal hemorrhage, PEF and AKI. Six months post-transplant she developed worsening hematuria, proteinuria with intractable hypertension (requiring five anti-hypertensive medications). She was started on hemodialysis. Investigations revealed TA-TMA (Table 1). There was minimal response to discontinuation of cyclosporine, therapeutic plasma exchange and basiliximab. A renal biopsy performed showed acute, severe thrombotic microangiopathy (Figure 1) with C4d deposits in glomerular capillary loops and arterioles (not shown). A month later she presented with seizures and brain MRI revealed diffuse white matter abnormality consistent with severe TMA and therefore she was started on eculizumab therapy and received 900 mg/week (weeks 1-4) followed by 900 mg/2 weeks (weeks 5- 21). Hematological response was seen after 4 weeks of therapy. She had persistent nephrotic range proteinuria with no evidence of renal recovery. She progressed to CKD with HTN (requiring four anti-hypertensives) and remained dialysis dependent with an eGFR of 35ml/min/1.73m^2(pre-transplant eGFR 103ml/min/1.73m^2). She died 16 months post-transplant due to RSV-related respiratory failure and TA-TMA related acute on CKD.

Discussion

The vascular endothelium is a dynamic surface, which upon exposure to various stimuli, undergoes changes in gene expression and participates in inflammatory reactions, immunity and thrombosis [7]. Earlier studies in hemoglobinopathies have demonstrated an increase in the number of circulating endothelial cells (CECs) that express surface markers of endothelial activation and injury like soluble intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) [8,9].

Studies in hemoglobinopathies have shown that products of red cell breakdown: plasma free hemoglobin, heme and arginase-1 disrupt endothelial function and drive oxidative inflammatory stress known as erythrocyte damage associated molecular patterns (eDAMPs) [3,4]. These eDAMPs are implicated in the pathogenesis of vascular related complications associated with hemoglobinopathies. Interestingly, there are several reports of SCD patients (HbSS, HbSC and HbSβ0) in non-transplant setting presenting with TTP following SCD crisis [10-11]. These patients had normal ADAMTS13 levels and responded to therapeutic plasma exchange. It is unknown if pre-existing vascular dysfunction influences onset and severity of TA-TMA in patients with hemoglobinopathies undergoing HCT.

TA-TMA is a multisystem disease characterized by vascular injury, variable clinical presentation and predominant renal involvement [12]. Diagnosis is based on clinical criteria published by Blood and Marrow Clinical Trial Network [13]. These criteria may be absent in early stages of TA-TMA as noted in our patients, leading to diagnostic confusion especially in the presence of co-existing morbidities such as GvHD and infections [14].

HTN, elevated lactate dehydrogenase (LDH), and proteinuria are early markers for TA-TMA [14]. PEF is seen in 30% patients with TA-TMA and may be the only manifestation as seen in patient 1 [15]. During transplant conditioning regimens,

opportunistic infections, GvHD and immunosuppressive agents initiate and aggravate endothelial damage, the pathological hallmark of TA-TMA [12]. Increased levels of markers of endothelial damage like von Willebrand factor antigen, thrombomodulin, plasminogen activator inhibitor 1, ICAM-1, and CECs have been reported in TA-TMA [12,16].

Recent studies have shown complement activation and dysregulation in the pathogenesis of TA-TMA [17,18]. Eculizumab a monoclonal antibody against the complement component C5, blocks formation of membrane attack complex and is an effective treatment for TA-TMA Normalization of intravascular hemolysis indices and proteinuria to non-nephrotic range as originally described by Jodele et al was used to guide eculizumab therapy in both patients [19]. Additionally, in patient1, hemolytic complement activity: CH50 (a surrogate marker of complement activation) levels were monitored twice weekly during therapy. Levels were kept $\leq$ 4 complement activity enzyme (CAE) units as this has been shown to correlate with therapeutic eculizumab levels of > 99µg/ml [19]. Therapy was tolerated and there were no infusion related side effects or severe bacterial infections in both patients. We recommend the recent dosing schedule suggested by Jodele et al utilizing CH50 and sC5-9 b levels to monitor response and guide frequency and duration of eculizumab therapy [20].

While both our patients had severe TA-TMA with a poor renal response, it is possible that eculizumab therapy prior to overt clinical manifestations may have prevented CKD. As evidenced in our first patient, long term monitoring of kidney functions with control of HTN is essential, given the potential for some renal recovery.

Conclusion

We conclude that patients with hemoglobinopathies may be pre-disposed to TA-TMA, given pre-existing endothelial dysfunction and subsequent vascular injury sustained

during HCT. Future clinical trials utilizing HCT for hemoglobinopathies should evaluate the utility of early markers of endothelial damage to diagnose TA-TMA. This will help to determine the role of pre-emptive eculizumab therapy prior to the onset of overt clinical manifestations and prevention of CKD.

Conflicts of Interest: None

References:

1. Butthep P, Nuchprayoon I, Futrakul N. Endothelial injury and altered hemodynamics in thalassemia. Clin.Hemorheol.Microcirc. 2004;31:287-293.

2. Hahalis G, Kremastinos DT, Terzis G, et al. Global vasomotor dysfunction and accelerated vascular aging in beta-thalassemia major. Atherosclerosis. 2008;198:448-457.

3. Kassim AA, DeBaun MR. Sickle cell disease, vasculopathy, and therapeutics. Annu.Rev.Med. 2013;64:451-466.

4. Mendonca R, Silveira AA, Conran N. Red cell DAMPs and inflammation. Inflamm.Res. 2016;65:665-678.

5. Laskin BL, Goebel J, Davies SM, Jodele S. Small vessels, big trouble in the kidneys and beyond: hematopoietic stem cell transplantation-associated thrombotic microangiopathy. Blood. 2011;118:1452-1462.

6. Glezerman IG, Jhaveri KD, Watson TH, et al. Chronic kidney disease, thrombotic microangiopathy, and hypertension following T cell-depleted hematopoietic stem cell transplantation. Biol.Blood Marrow Transplant. 2010;16:976-984.

7. Mantovani A, Bussolino F, Dejana E. Cytokine regulation of endothelial cell function. FASEB J. 1992;6:2591-2599.

8. Butthep P, Rummavas S, Wisedpanichkij R, Jindadamrongwech S, Fucharoen S, Bunyaratvej A. Increased circulating activated endothelial cells, vascular endothelial growth factor, and tumor necrosis factor in thalassemia. Am.J.Hematol. 2002;70:100-106.

9. Solovey A, Lin Y, Browne P, Choong S, Wayner E, Hebbel RP. Circulating activated endothelial cells in sickle cell anemia. N.Engl.J.Med. 1997;337:1584-1590.

10. Shome DK, Ramadorai P, Al-Ajmi A, Ali F, Malik N. Thrombotic microangiopathy in sickle cell disease crisis. Ann.Hematol. 2013;92:509-515.

11. Shelat SG. Thrombotic thrombocytopenic purpura and sickle cell crisis. Clin.Appl.Thromb.Hemost. 2010;16:224-227.

12. Batts ED, Lazarus HM. Diagnosis and treatment of transplantation-associated thrombotic microangiopathy: real progress or are we still waiting?. Bone Marrow Transplant. 2007;40:709-719.

13. Ruutu T, Barosi G, Benjamin RJ, et al. Diagnostic criteria for hematopoietic stem cell transplant-associated microangiopathy: results of a consensus process by an International Working Group. Haematologica. 2007;92:95-100.

14. Jodele S, Davies SM, Lane A, et al. Diagnostic and risk criteria for HSCT-associated thrombotic microangiopathy: a study in children and young adults. Blood. 2014;124:645-653.

15. Lerner D, Dandoy C, Hirsch R, Laskin B, Davies SM, Jodele S. Pericardial effusion in pediatric SCT recipients with thrombotic microangiopathy. Bone Marrow Transplant. 2014;49:862-863.

16. Erdbruegger U, Woywodt A, Kirsch T, Haller H, Haubitz M. Circulating endothelial cells as a prognostic marker in thrombotic microangiopathy. Am.J.Kidney Dis. 2006;48:564-570.

17. Jodele S, Licht C, Goebel J, et al. Abnormalities in the alternative pathway of complement in children with hematopoietic stem cell transplant-associated thrombotic microangiopathy. Blood. 2013;122:2003-2007.

18. Jodele S, Zhang K, Zou F, et al. The genetic fingerprint of susceptibility for transplant-associated thrombotic microangiopathy. Blood. 2016;127:989-996.

19. Jodele S, Fukuda T, Vinks A, et al. Eculizumab therapy in children with severe hematopoietic stem cell transplantation-associated thrombotic microangiopathy. Biol.Blood Marrow Transplant. 2014;20:518-525.

20. Jodele S, Fukuda T, Mizuno K, et al. Variable Eculizumab Clearance Requires Pharmacodynamic Monitoring to Optimize Therapy for Thrombotic Microangiopathy after Hematopoietic Stem Cell Transplantation. Biol.Blood Marrow Transplant. 2016;22:307-315.

LEGEND List:

Figure. 1. Renal Biopsy: Glomeruli showing evidence of acute TMA in glomerular capillary loops with marked vascular congestion, mesangiolysis and erythrocyte fragments.

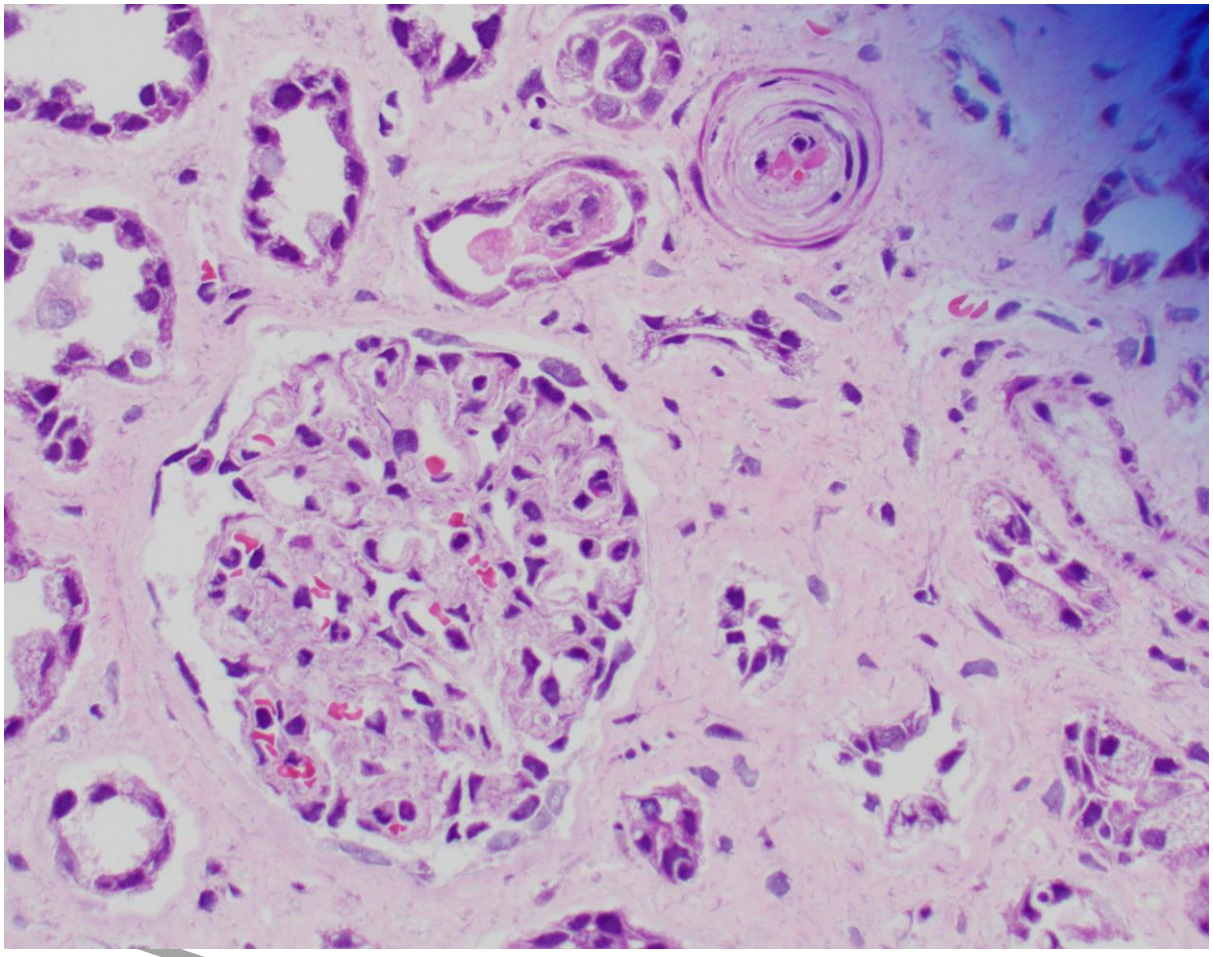

TABLE 1: Laboratory Values at Diagnosis

Test	Patient 1	Patient 2
LDH	2926 (350 - 850 U/L)	1121 (140 - 280 U/L)
Hemoglobin	8 (11.5 - 13.5 gm/dl)	7.4 (11.9 - 15 gm/dl)
Reticulocyte count	6 (0.4 - 1.8%)	4.6 (0.5 - 2%)
Platelet counts	114,000 cells/mm^3	42,000 cells/mm^3
Haptoglobin	30 (33 - 171 mg/dl)	<20 (30 - 200 mg/dl)
Peripheral shistocytes	Rare to absent	3+

Urine protein	324 (< 100 mg/dl)	300 (<100 mg/dl)
Urine protein creatinine ratio	14.14 (0.19 mg of protein/mg of creatinine)	20.7 (0.2 mg of protein/mg of creatinine)
sC5-9b at diagnosis	139 (74 - 244 ng/ml)	Not tested
ADAMTS13 level	70 (>66%)	65 (>66%)
Factor B	21.1 (13.3 - 31.5 mg/dl)	25.8 (12.7-27.8 mg/dl)
Factor I	3.7 (2.4 - 4.9 mg/dl)	4.95 (2.9 - 5.8 mg/dl)
Factor H	65.6 (37- 68 mg/dl)	32.1 (16 - 41 mg/dl)
Factor H antibody	Negative	Negative
C3	178 (86 - 184 mg/dl)	112 (90 - 180 mg/dl)
C4	56 (16 - 59 mg/dl)	Not tested
CH50	221 (101- 300 CAE U/ml)	201 (60 – 144 CAEU/ml)
Renal biopsy	No	Yes, TMA changes

224 LDH, Lactate Dehydrogenase; CAE units, Complement Activity enzyme units; Normal
225 values for age are in parenthesis.

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