

REVIEW

Thrombotic complications associated with stem cell transplantation

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ARTICLE INFO

Keywords:
Thrombosis
Endothelial cells
SCT
VOD
TMA
DAH

ABSTRACT

Thrombotic complications are common in stem cell transplantation (SCT) recipients and endothelial cell injury is a dominant contributing factor to the hemostatic impairments. Endothelial cells line the vascular bed and each vascular bed has a unique structural and functional properties. Therefore, understanding of these properties may hold important clues to site-specific diagnostics and therapeutics. The two most common thrombotic manifestations related to SCT, veno-occlusive disease (VOD) and thrombotic microangiopathy (TMA), are characterized by small vessel thrombosis in the microcirculation. In diffuse alveolar hemorrhage (DAH), although the clinical presentation is hemorrhagic, autopsy findings and mice experiments imply a thrombotic etiology. In the present review, the pathogenesis and treatment options of these three microcirculation thromboses are discussed.

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1. Introduction

Thrombotic complications are a major cause of morbidity and mortality in stem cell transplantation (SCT) recipients. Endothelial cell injury is a dominant contributing factor to the hemostatic impairments related to administration of chemotherapy¹ and growth factors,¹ use of intravenous catheters, and graft-versus-host reaction.² As bone marrow endothelial progenitor cells (EPCs) are the source for endothelial regeneration, the ability to repair the endothelial damage during SCT is limited. Similar to the reduced ability of hematopoietic stem cells to reconstitute blood cells, EPC proliferation is also arrested under the influence of transplant preparative regimens. Profound thrombocytopenia is known to be a major limiting factor to adequate thrombo-prophylaxis. Refractoriness or severe reactions to platelet transfusions, common in patients previously exposed to blood product transfusions during chemotherapy, predispose them to life-threatening bleeding events.

Endothelium-derived anticoagulant and procoagulant molecules are not uniformly expressed in the vasculature. For example, TFPI is predominantly expressed in the capillary endothelium, EPCR — in large veins and arteries, endothelial NO synthase (eNOS) — on the arterial side of the circulation, VWF prevails in veins and TM in blood vessels of every caliber in all organs apart from the brain.^{14–16} These data suggest that ECs originating from different sites of the vascular tree employ site-specific formulas of procoagulants and anticoagulants to balance local hemostasis.¹¹ The two most common thrombotic manifestations

related to SCT, veno-occlusive disease (VOD) and thrombotic microangiopathy (TMA), are characterized by small vessel thrombosis in the microcirculation.

The present review will deal primarily with thrombotic conditions that are specific for SCT, including VOD and TMA. Diffuse alveolar hemorrhage (DAH), although clinically manifesting with bleeding, also potentially has a thrombotic etiology. Illness-related major vein thrombosis (e.g., deep vein thrombosis, pulmonary emboli) occurs in SCT patients as well as in many other conditions of hospitalized patients and are not specific.

2. Hepatic veno-occlusive disease (VOD)

VOD develops in 1–22% of patients after SCT and ranges in severity from a mild, reversible disease to a severe syndrome associated with multi-organ failure and death. It is characterized by painful hepatomegaly, jaundice, ascites, fluid retention and weight gain,^{3–6} with onset typically prior to day 35 after stem cell reinfusion.^{3–6} The incidence and severity of VOD can be influenced by differences in patient characteristics, conditioning regimens, and source of graft, with allogeneic transplantation conferring a 3-fold increase in risk compared to autologous transplantation.^{7,8}

3. Pathogenesis

Hepatic sinusoidal ECs are discontinuous and lack an organized basement membrane.⁹ The lack of basement membrane generates a space between sinusoidal ECs and hepatocytes called the space of Disse. Blood flow velocity in the sinusoids has been estimated to be 400–450 $\mu\text{m/s}$, compared with 500–1000 $\mu\text{m/s}$ in “true capillaries,” thus prolonging interactions between blood and sinusoidal ECs and

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promoting filtration. In addition, the blood in the sinuses near the centrilobular space is oxygen-poor, disabling effective endothelial cell injury repair due to relative hypoxia.⁹ The conditions of lacking basement membrane enabling rapid endothelial cells dissection, slow down blood flow causing prolonged exposure to chemotherapy and stasis, in addition to poor oxygenation, and predispose the centrilobular region to thrombosis.

Sinusoidal obstructive syndrome, also known as hepatic VOD, occurs most commonly in response to conditioning regimens (chemotherapy with or without irradiation) used in bone marrow transplantation. The first lesions appear in the sinusoids. Experimental models suggest that exposure to toxins results in swelling and rounding up of sinusoidal ECs, especially in the centrilobular area that has the poorest oxygenation and is relatively more sensitive to ischemic injury, after which red blood cells enter the space of Disse.¹⁰ Here blood flow essentially dissects the sinusoidal endothelial lining away from the underlying parenchymal cells, resulting in embolization of sinusoidal ECs and occlusion of the downstream venous vessel.¹⁰ Subsequent changes include deposition of fibrin within sinusoids and veins, fibroblast cell proliferation and collagen accumulation in the extracellular matrix.^{11–13} As the process of venous microthrombosis, ischemia, and fibrosis advances, widespread zonal disruption leads to portal hypertension, hepato-renal syndrome, multi-organ failure, and death.¹⁴

Injury to sinusoidal endothelial cells caused by high-dose alkylating agents appears to be the primary event in the pathogenesis of VOD.^{15–17} Detoxification of these chemotherapy agents in the liver is mainly mediated by cytochrome P450 and glutathione-S-transferase enzymes, which are present at high concentrations within the centrilobular area.¹⁸ Depletion of glutathione stores predisposes hepatocytes to necrosis, whereas addition of glutathione protects hepatocytes from high-dose alkylator injury.^{19,20}

Several endothelial injury markers and adhesion molecules are upregulated in patients with VOD, including plasma TM, P- and E-selectins, TFPI, soluble tissue factor, and PAI-1.^{21,22}

In vitro studies show that endothelial PAI-1 production is triggered by cytokine transforming growth factor beta (TGF- β) released from activated platelets²³ and elevated pre-transplant plasma levels of TGF- β have been associated with the development of hepatic VOD.²⁴ Additionally, other pro-inflammatory cytokines such as tumor necrosis factor (TNF- α), interleukin (IL)-6, IL-8, and IL-1 β can also contribute to the initial endothelial injury in VOD.^{25,26}

The role of thrombophilic factors in the pathogenesis of thrombotic complications in SCT is unclear. In a small retrospective study the prothrombin gene 20210G-A mutation was found to be a predisposing factor for VOD,²⁷ and a prospective study in children revealed a strong association between factor V Leiden mutation and VOD.²⁸ More studies are warranted to confirm the association between thrombophilia and VOD.

4. Therapy

The use of pharmacokinetics to monitor chemotherapeutic drug levels to minimize hepatic injury is the most established practice in VOD prevention.²⁹ Prophylactic administration of ursodeoxycholic acid, a hydrophilic water-soluble bile acid, has been studied in randomized placebo controlled trials, some showing a statistically significant benefit in patients predicted to be at a high risk of VOD.^{30–32} However, a large phase III study failed to demonstrate significant benefit of this approach.³³ The role of heparin anticoagulation, alone or in combination with other agents, in VOD prophylaxis has been assessed in only one randomized study, which reported a beneficial effect of low-dose continuous heparin infusion.³⁴ LMWH is relatively safe and may have some impact on VOD prevention,^{35–38} but well-designed studies are needed to confirm these results.

There is no approved therapy for established hepatic VOD. Current approaches focus on supportive care and anticoagulant or fibrinolytic drugs,^{34,35,39–41} based on pathological findings of hepatic venules or sinusoids obliteration by fibrin.^{4,12,13} The major disadvantage of exposure to heparin or recombinant tissue-type plasminogen activator (rt-PA) is the high risk of life-threatening bleeding in patients with concurrent thrombocytopenia.^{40,42}

Defibrotide, a single-stranded polydeoxyribonucleotide that exerts anti-thrombotic activity by binding to vascular endothelium, has shown promising results in VOD management.^{43–45} Defibrotide up-regulates the endothelial release of prostacyclin (PG I₂), prostaglandin E₂, TM, and t-PA,^{46–48} and decreases thrombin generation, tissue factor expression, PAI-1 release, and endothelin activity.^{46,49} Defibrotide exerts no significant effect on systemic coagulation,⁵⁰ but has an anti-angiogenic potential in endothelial cells and in an animal model.⁵¹ Preclinical studies have demonstrated profibrinolytic effects and inhibition of fibrin deposition, with selective activity in small vessels.⁵² Initial clinical reports of defibrotide for severe VOD recorded complete resolution in 36–42% of patients, with most individuals surviving beyond day 100.^{43,44,53} In a recent randomized phase II dose-finding trial in adult and pediatric patients, defibrotide 25 mg/kg/day was selected for forthcoming phase III trials.⁵⁴ Pre-emptive antithrombin replacement and combined antithrombin/defibrotide therapy allowed excellent remission and survival rates in a prospective case series study of pediatric SCT patients.⁵⁵ In another report on 58 adults undergoing SCT, no patient developed VOD following the use of defibrotide prophylaxis (without concurrent heparin).⁵⁶ Cappelli et al. reported on 57 children with beta thalassemia at a very high risk for developing VOD (liver fibrosis, iron overload, hepatitis C virus infections, busulphan-based conditioning), and only one patient developed VOD, after discontinuation of defibrotide 6 days prior to VOD onset.⁵⁷ To date, no phase III randomized studies have been published on the use of defibrotide as prophylaxis or treatment of VOD.

5. Transplantation-related thrombotic microangiopathy (TMA)

The incidence of TMA following allogeneic and autologous transplantation is 0.5–15% and 0.1–0.25%, respectively.^{58–60} The diagnosis is principally based on the presence of thrombocytopenia and microangiopathic hemolytic anemia in the absence of an alternative clinically apparent etiology.^{61,62} Similar to idiopathic thrombotic thrombocytopenia purpura (TTP), transplantation-related TMA is associated with renal dysfunction and neurologic complications.⁶² However, since both thrombocytopenia and fragmentation of red blood cells are extremely common after SCT, and renal dysfunction and neurologic complications can occur secondary to a diverse range of etiologies, definitive diagnosis of transplantation-related TMA is often uncertain.^{63,64} Transplantation-related TMA can be attributed to extensive prior therapy, GVHD, reactivation of cytomegalovirus and drugs (cyclosporine-A, tacrolimus).^{60,65,66} Although transplantation-related TMA is associated with a shorter survival due to multi-factorial causes, the exact prognosis is difficult to assess.⁶⁷

6. Pathogenesis and therapy

Elevated VWF antigen levels found in patients with TMA are more likely the result of diffuse endothelial injury and not due to severe VWF-cleaving protease deficiency.^{60,68} Cyclosporine-A is cytotoxic to cultured endothelial cells at concentrations similar to the peak plasma levels achieved in vivo.⁶⁹ During episodes of cyclosporine-A induced microangiopathy, plasma levels of VWF and endothelin are elevated,⁷⁰ as well as those of prostacyclin and thromboxane A₂ released by endothelial cells.⁶⁹ In addition, cyclosporine-A enhances platelet aggregation and platelet thromboxane A₂ release. Platelet hyperaggregability in renal allograft patients on long-term cyclosporine-A therapy reverts

towards normal following the replacement of the drug with azathioprine.⁷⁷ In patients with transplant-related TMA who received tacrolimus, a rise in serum levels of endothelin and several cytokines (IL-10, IL-12, TNF- α , interferon- γ) occurs, consistent with endothelial cell injury.⁷¹

To date, the optimal management of transplantation-related TMA has not been established. Certainly, any suspected offending drug should be discontinued. Because of clinical similarity to idiopathic TTP, treatment-related TMA has been managed with plasma exchange, albeit with limited efficacy and relatively low response rates of <20–50% compared with idiopathic TTP (80%).^{61,64,72} Other treatment modalities, such as defibrotide or immunoglobulin G, have been attempted with variable success.^{73,74}

7. Diffuse alveolar hemorrhage (DAH)

The reported incidence of DAH varies between 1 and 21% in auto-grafted and between 2 and 17% in allografted patients, suggesting that the type of transplantation does not play a major role in this pathology.^{75–79} Clinical presentation of DAH includes hypoxemia, pulmonary infiltrates, and progressive bloody alveolar lavage. A documented associated infectious cause was reported in 50–60% of the patients.⁸⁰ The probability of a 60-day survival from the onset of hemorrhage was 16% for the DAH and 32% for the infection-associated alveolar hemorrhage group.⁸⁰ Allogeneic SCT using reduced intensity conditioning (RIC) has lower morbidity and mortality compared to transplantation using myeloablative conditioning (MAC), although alveolar hemorrhage occurring in 18/206 (8%) and 85/1112 (7%, $p = 0.56$), respectively, indicates that reduction in the intensity of a preparative regimen does not prevent transplantation-related DAH.⁸¹ While white blood cell (WBC) recovery and renal insufficiency are associated with DAH, prolonged prothrombin time (PT), partial thromboplastin time (PTT), or low platelet counts are not.^{82,83} Although most patients have thrombocytopenia, the condition is not corrected with platelet transfusion.⁸³

8. Pathogenesis and therapy

Autopsies of SCT recipients with acute hemorrhagic pulmonary edema show intra-alveolar and, to a lesser extent, interstitial collections of edema, erythrocytes and fibrin, without hyaline membranes, pneumocyte changes, interstitial mononuclear cell infiltration or fibrosis.⁸⁴ Thus, although the clinical presentation is hemorrhagic, autopsy findings with fibrin deposition imply a thrombotic etiology (Fig. 1A and B). The incidence of DAH is high in SCT recipients with acute GVHD.⁸⁵ In addition to toxicity of the GVHD therapy, antigen-specific injury of endothelium may be a contributing factor to the development of DAH.⁸⁶ Mice receiving bone marrow T lymphocytes develop alveolitis, characterized by alveolar hemorrhage, increased alveolar leukocytes, platelet microthrombi, and damage to endothelial cells during the acute phase of GVHD, supporting an endothelial injury and thrombotic mechanism.² The use of dimethyl sulfoxide for cryopreservation of blood stem cells has been implicated in causing damage to the alveolar endothelial lining, thereby leading to the development of DAH.^{78,87} Hematopoietic growth factors may also play a role in worsening the alveolar damage and capillary leakage by increasing neutrophil infiltration into the lungs.^{88,89} It is speculated that damage to alveolar capillary endothelial membranes begins during preparative chemotherapy or total body irradiation, resulting in release of inflammatory mediators,⁹⁰ a response that may be amplified after the release of endotoxin into circulation from an inflamed gastro-intestinal tract.⁹⁰

While capillary diameter in most organs is about 10 μm , it is about 8 μm in the pulmonary bed, enforcing deformability of granulocytes and monocytes which are 10–17 μm in diameter.⁹¹ DAH typically occurs in parallel to WBC recovery.^{82,83} Impaired ability of WBCs to

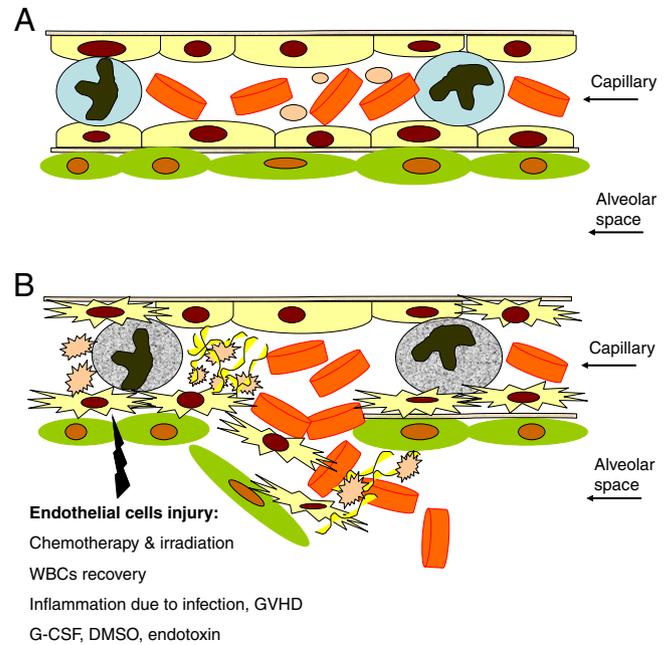


Fig. 1. Proposed mechanism of DAH. (A) Normal capillary and alveolar space. Pulmonary capillary bed has small diameter, hardly enabling WBCs passage. (B) Alveolar capillary endothelial cell injury is the primary insult. WBC recovery exacerbates the thrombotic potential due to the large diameter of neutrophils and tissue factor exposure during inflammation, infection and G-CSF treatment. Injured endothelium activates platelets and coagulation factors to form fibrin. The thrombotic capillary ruptures and red blood cells enter the alveolar space resulting in alveolar hemorrhage.

transmigrate lung ECs, as was shown in the mice model of DAH,² together with endothelial damage, may engender local alveolar capillary endothelial thrombosis resulting in endothelial damage and hemorrhage (Fig. 1B).

There are no prospective, randomized trials addressing the treatment of DAH in SCT recipients. Given that the pathogenesis of DAH is considered to be an inflammatory response and based on anecdotal experience and retrospective studies, SCT recipients with DAH are treated with systemic corticosteroids.^{76,78,90,92} Fresh frozen plasma transfusion and plasmapheresis have been tried in one study with inconclusive results.⁹³ Paucity of reports on the off-label use of recombinant factor VIIa (rFVIIa) in SCT recipients with DAH precludes drawing definitive conclusions regarding the efficacy of this therapy.^{94,95} In a phase II multicenter trial in SCT patients with major bleeding a total dose of 280–1120 $\mu\text{g}/\text{kg}$ rFVIIa was used,⁹⁶ with regimens of 40, 80, or 160 $\mu\text{g}/\text{day}$ administered in seven successive doses every 6 h for 36 h. In this study, 7 patients out of 100 suffered from DAH, 6 thromboembolic events were observed in the treated group, and the trial did not show a significant benefit of rFVIIa in the setting of SCT-related severe bleeding.⁹⁶ The use of antifibrinolytic agents, such as tranexamic acid and aprotinin, is not well documented. Solomonov et al. reported on six patients with significant hemoptysis, two of whom bled during bronchoscopy biopsy and four with spontaneous bleeding, treated successfully with local tranexamic acid without adverse events.⁹⁷

9. Conclusions

Hemostatic complications are known to cause high morbidity and mortality in SCT patients. The use of more intensive conditioning regimens and mismatched donors increases the incidence of thrombosis as well as bleeding. Due to the possibility that DAH could also be a thrombotic event, the three typical hemostatic complications in SCT, namely, VOD, TMA and DAH, may be manifestations of thrombosis in the microcirculation. Proliferation of EPCs, being the source of

endothelial cells repair is attenuated under the effect of SCT conditioning regimen, rendering the vascular bed to vulnerability. Ex-vivo expansion of EPCs as a source of endothelial cells replacement is extensively investigated.⁹⁸ New strategies of using embryonic stem cells differentiated toward pericytes⁹⁹ or injection of growth factors such as platelet-derived growth factor (PDGF),¹⁰⁰ are intriguing new modalities to overcome microcirculation injury.

Conflict of interest statement

No conflicts of interest to declare.

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