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Thrombotic Microangiopathy in Haematopoietic Stem Cell Transplantation

Diagnosis and Treatment

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Abstract

Each year in the US, more than 10 000 patients benefit from allogeneic haematopoietic stem cell transplantation (HSCT), a modality that offers an excellent chance of eradicating malignancy but confers a higher risk of treatment-related mortality. An uncommon but devastating consequence of HSCT is transplantation-associated thrombotic microangiopathy (TA-TMA). The incidence of TA-TMA ranges from 0.5% to 76%, with a mortality rate of 60–90% despite treatment. Although there appears to be a consistent treatment approach to idiopathic thrombotic thrombocytopenic purpura (TTP) using plasma exchange, corticosteroids and rituximab, the treatment strategies for TA-TMA are perplexing, in part, because the literature regarding this complex condition does not provide true consensus for incidence, aetiology, diagnostic criteria, classification and optimal therapy. The classic definition of idiopathic TTP includes schistocytes on the peripheral blood smear, thrombocytopenia and increased serum lactate dehydrogenase. Classic idiopathic TTP has been attributed to deficient activity of the metalloproteinase

responsible for cleaving ultra-large von Willebrand factor multimers. This protease is a member of the 'a disintegrin and metalloprotease with thrombospondin type 1 motif' family and is subsequently named ADAMTS-13. Severely deficient ADAMTS-13 activity (<5% of normal) is associated with idiopathic TTP in 33–100% of patients. In constrast to the pathophysiology of idiopathic TTP, patients with TA-TMA have >5% ADAMTS-13 serum activity. These data may explain why plasma exchange, a standard treatment modality for idiopathic TTP that restores ADAMTS-13 activity, is not effective in TA-TMA. TA-TMA has a multifactorial aetiology of endothelial damage induced by intensive conditioning therapy, irradiation, immunosuppressants, infection and graft-versus-host disease. Treatment consists of substituting calcineurin inhibitors with an alternative immunosuppressive agent that possesses another mode of action. One candidate may be daclizumab, especially in those with mild to moderate TMA. Rituximab therapy or the addition of defibrotide may also be beneficial. In general, plasma exchange is not recommended.

Each year in the US, more than 10 000 patients benefit from allogeneic haematopoietic stem cell transplantation (HSCT).[1] In the past, bone marrow was the standard source of haematopoietic cells but peripheral blood stem cells have nearly supplanted marrow as the graft source. Three-quarters of bone marrow transplant (BMT) or peripheral blood stem cell transplant (PBSCT) procedures are performed for patients with leukaemias, lymphomas or other types of haematological malignancies,[1] but there may be an expanding role for PBSCT in diseases such as solid tumours (e.g. renal cell carcinomas) as well as nonmalignant diseases such as sickle cell disease and autoimmune disorders (e.g. systemic lupus erythematosus).[2] Non-myeloblative PBSCT conditioning regimens are associated with a reduced treatment-related mortality and enable treatment for older adults with diseases such as myelodysplastic syndrome, acute myelogenous leukaemia and non-Hodgkin's lymphoma. In 2007, 35% of National Donor Marrow Program transplant recipients were aged ≥50 years.[1] The increased use of PBSCT is attributed to faster haematopoietic recovery of neutrophils and platelets,[3] and easier collection of cells. A total of 30% of the patients receive their transplant from family members (known as matched related donor), whereas the remaining 70% receive unrelated donor or umbilical cord blood units (matched unrelated donor).[1] Allogeneic transplantation offers a good chance of eradicating malignancy but confers a higher risk of treatment-related mortality.

An uncommon but devastating consequence of HSCT is transplantation-associated thrombotic microangiopathy (TA-TMA), also known as transplant-associated microangiopathy, a thrombotic thrombocytopenic purpura (TTP) syndrome manifest as a microangiopathic haemolytic anaemia (MAHA). The literature regarding this complex condition does not provide true consensus for many aspects including incidence, aetiology, diagnostic criteria, classification and optimal therapy. The reported incidence of TA-TMA has varied enormously owing partly to the use of differing diagnostic criteria, ranging from 0.5% to 76% with high mortality rates of 60–90%[4-15] despite treatment.

For the specific entity of idiopathic TTP, the incidence is 4.46 cases per million population.[16] In some patients, abnormalities of the metalloproteinase responsible for cleaving ultra-large von Willebrand factor (vWF) multimers, a member of the 'a disintegrin and metalloprotease with thrombospondin type 1 motif' family subsequently named ADAMTS-13, appear to be the aetiology (see section 1). The profound ADAMTS-13 deficiency of circulating enzyme is usually caused by autoantibody formation, while in a minority, a congenital mutation accounts for lack of enzyme.

The mechanism of the MAHA is not completely known, but a recent publication suggests that secreted vWF adheres to endothelial cells via its $\alpha_v\beta_3$ integrin and forms long strings, which, under high shear, may result in red blood cell fragmentation within the intravascular compartment.[17] Many haematologists now advocate a diagnosis of chronic relapsing or acute idiopathic TTP based on the dyad of MAHA and thrombocytopenia. When untreated, mortality rates for this syndrome range from 60% to 90%. With institution of plasma exchange or infusion, mortality can be lowered to <25%.[18]

Although there appears to be a more consistent treatment approach to idiopathic TTP (plasma exchange and infusion of fresh frozen plasma, corticosteroids and rituximab), the treatment strategies for TA-TMA are not consistent because of a lack of aetiological understanding of these transplant-related syndromes. A systematic review of 35 articles describing 447 TA-TMA patients revealed that reports in 11 of 35 autopsied patients had no evidence of TMA.[5] The difficulty in identifying an aetiology for TA-TMA arises from the significant co-morbidity associated with transplantation itself and differences in pathophysiology of TA-TMA versus idiopathic TTP. In this review, we attempt to clarify the definition of TA-TMA, discuss the pathophysiology of TA-TMA and suggest treatment alternatives.

1. Pathophysiology

TA-TMA encompasses primary processes such as idiopathic TTP and haemolytic-uraemic syndrome (HUS). The classic description of idiopathic TTP includes schistocytes on the peripheral blood smear, thrombocytopenia and increased serum lactate dehydrogenase (LDH). Idiopathic TTP has been attributed to deficient activity of the metalloproteinase responsible for cleaving ultra-large vWF multimers.[19] Severely deficient ADAMTS-13 activity (<5% of normal) is associated with TTP in 33–100% of patients.[15,20] Insufficient ADAMTS-13 activity may result from either an inhibitory antibody[21] (most commonly) or rarely, a true deficiency of the enzyme.[22] Familial forms of TTP (Upshaw-Schulman syndrome), inherited in an autosomal recessive manner,[19] typically manifest during the neonatal period but can also uncommonly present in adulthood.[23] Upshaw-Schulman patients lack the vWF-cleaving protease activity and do not have demonstrable antibodies to ADAMTS-13.[19,21]

Autoimmune inhibition of ADAMTS-13 accounts for approximately 70–80% of idiopathic TTP.[24] Shear stress on an injured vessel wall causes the large vWF multimers to unfold. This change in configuration provides the substrate on the endothelium for platelet rolling, adhesion and aggregation. Bloodstream ADAMTS-13 cleaves the exposed, unfolded vWF at specific sites forming smaller vWF multimers. The multimers do not avidly bind platelets and generate microvascular platelet thrombosis.[25] In ADAMTS-13 deficiency, platelets form more aggregates and partially occlusive vWF-platelet aggregates or strands of uncleaved vWF shear red blood cells, producing a MAHA.[24,26]

The classic syndrome of TTP regularly involves CNS and renal dysfunction, and spares organs such as liver or lung. It is postulated that microvascular endothelial cell apoptosis is correlated with TTP. Plasma retrieved from patients with idiopathic TTP/HUS induce apoptosis of microvascular endothelial cells derived from kidney, brain[27] and skin. Eremina et al.[28] have shown that renal microvascularity is particularly susceptible to thrombotic microangiopathy, as demonstrated by their recent report on the effects of inhibition of vascular endothelial growth factor. Conversely, apoptosis did not occur in endothelial cultures derived from large vessels or lungs, demonstrating the tissue-specific cells associated with TTP.[29] Measurement of ADAMTS-13 activity correlates well with anti-ADAMTS-13 autoantibody concentration and a fall in antibody preceded a rise in ADAMTS-13 activity in 5 out of 6 patients with TTP in one study.[30]

In contrast to the pathophysiology of TTP, patients with TA-TMA have >5% ADAMTS-13 serum activity.[31-34] Therefore, the primary aetiology is not ADAMTS-13 deficiency. These data may explain why plasma exchange, a standard

treatment modality for TTP that restores ADAMTS-13 activity, is not effective in TA-TMA.[31] TA-TMA is most likely to have a multifactorial aetiology of endothelial damage induced by intensive conditioning therapy, irradiation, immunosuppressants, infection and graft-versus-host disease (GVHD).[4,32-37]

In TMA, several factors contribute to the development of endothelial cell damage. Endothelial microparticles may lead to platelet activation inducing microthrombosis and predisposing to TMA.[27,38-43] Endothelial microparticles expressing markers of endothelial activation (CD62 and annexin V) are increased in the setting of acute GVHD,[44] and increased circulating platelet- and monocyte-derived microparticles have been observed in one case of TA-TMA.[45] Unlike patients with idiopathic TTP, TA-TMA patients lack endothelial prostacyclin (PGI_2) release but have elevated levels of vWF antigen with normal vWF multimer patterns.[46] These findings are consistent with endothelial cell injury. Evidence for such an assessment include recognition of elevated thrombomodulin, plasminogen activator inhibitor (PAI)-1,[47] soluble intercellular adhesion molecule-1,[48-50] interleukin (IL)-1, tumour necrosis factor (TNF)-α, interferon (IFN)-γ and IL-8.[47] Similar patterns of endothelial cell injury have been found in inflammatory disorders such as Rocky Mountain Spotted Fever[51] and transplant conditioning, and in the setting of acute GVHD[49] and hepatic veno-occlusive disease (VOD).[12,52-54]

2. Definition and Diagnosis

Currently, there are two sets of diagnostic criteria available to standardize diagnosis of TA-TMA for clinical trials. According to the International Working Group, TA-TMA must include the following: (i) increased percentage (>4%) of schistocytes in the blood; (ii) *de novo*, prolonged or progressive thrombocytopenia (platelet count less than 50×10^9 cells/L or a ≥50% decrease from previous counts); (iii) a sudden and persistent increase in serum LDH; (iv) a decrease in haemoglobin concentration or increased red blood cell transfusion requirement; and (v) a decrease in serum haptoglobin concentration.[55] The diagnostic criteria for TA-TMA proposed by the Blood and Marrow Transplant Clinical Trials Network Toxicity Committee are as follows: (i) red blood cell fragmentation and at least two schistocytes per high-power field on peripheral smear; (ii) concurrent increased serum LDH above institutional baseline; (iii) concurrent renal (doubling of serum creatinine from baseline or 50% decrease in creatinine clearance from baseline) and/or neurological dysfunction without other explanations; (iv) negative direct and indirect Coombs test results.[13]

Unlike idiopathic TTP, as noted in section 1, patients with TA-TMA have >5% ADAMTS-13 activity and normal vWF multimeric patterns.[16,31-34] Thus, analysis of vWF protease activity and vWF multimeric distribution may help distinguish between idiopathic TTP and TA-TMA.[56] Although these diagnostic criteria from these two groups are helpful, they are probably still too inclusive in their present form. For example, they should also exclude evidence of disseminated intravascular coagulation by requiring normal blood coagulation assays, negative D-dimer and negative direct antiglobulin tests, and evidence for increased platelet-associated immunoglobulin. Problems with such criteria are that the vast majority of patients in the post-transplant period have already acquired many of the diagnostic criteria components such as persistent anaemia, thrombocytopenia, fever, renal dysfunction and neurological abnormalities. Furthermore, other transplant-related syndromes, such as a thrombotic microangiopathic syndrome caused by total body irradiation and chemotherapy nephropathy, can mimic TA-TMA, which further complicates the diagnosis.[4,57]

3. Classification of Thrombotic Microangiopathy Syndromes

TMA encompasses primary processes such as idiopathic TTP and HUS, as well as secondary disorders including TA-TMA. Other secondary causes include autoimmune disorders such as systemic lupus erythematosus, systemic sclerosis, antiphospholipid antibody syndrome, accelerated

and malignant hypertension, various infectious diseases and metastatic carcinoma. TA-TMA is difficult to distinguish from other categories of TMA because of the possibility of overlapping aetiologies. TMAs can be classified by subdividing into TA-TMA transplant- versus non-transplantation-associated TMA, as shown in tables I and II. In HSCT patients, the non-transplantation aetiologies

Table I. Classification of non-transplantation-associated thrombotic microangiopathy[24,58]

Condition	Example	ADAMTS-13 level	Treatment
Idiopathic TTP	Severe deficiency of ADAMTS-13 (relapsed triggered by pregnancy, infection, surgery and pancreatitis).[16,58] One-third with no neurological findings. 80% recover. Relapse rate = 50%[58]	Deficient	Plasma exchange, immunosuppressives
Congenital TTP	Homozygous or compound heterozygous mutation of the ADAMTS-13 gene[19,23]	Deficient	FFP
Familial-acquired TTP	IgG autoantibodies inactivating ADAMTS-13[59]	Deficient	FFP, plasma exchange
Haemolytic uraemic syndrome (HUS)	HUS due to fibrin deposition, Shiga toxaemia (Shiga toxin-producing bacteria *Escherichia coli* O157:H7 [risk factors: White, female, adults with renal failure and severe neurological abnormalities]) Components of thrombi platelets and fibrin (instead of vWF as in TTP)[60]	Decreased at onset[61] or normal[16]	NS
Pregnancy	Pregnancy, pre-eclampsia, HELLP syndrome[24] (usually near term or postpartum period, subsequent pregnancies not affected[58])	Normal[22] or deficient[16,58]	Plasma exchange, immunosuppressives
Autoimmune/vascular disorders	SLE flare, rheumatoid arthritis, scleroderma cryoglobulinaemia (usually in young adult females, may include renal failure; chronic course, high mortality)	Normal[16]	Plasma exchange, immunosuppressives
Medications	Non-oncological medications: quinine (most common cause of acute immune mediated drug toxicity [risk factors: White, female, elderly, sudden onset of acute renal failure with fever, diarrhoea, liver disease, neutropenia[58]]), cocaine, HMG-CoA reductase inhibitors, heparin[24]	Normal[16]	Plasma exchange, immunosuppressives not helpful, removal of offending medication
	Thienopyrine derivatives (most common drugs associated with TTP): ticlopidine, clopidogrel. If TTP was developed after 2 wk of therapy, plasma exchange increased likelihood of survival (84% vs 46%; $p < 0.003$). If before 2 wk, no benefit[62]	Deficient ADAMTS-13, increased IgG inhibitors[63]	Plasma exchange, removal of offending medication
DIC	Simultaneous activation of thrombin and plasma recognized by the presence of plasmin-cleaved, insoluble cross-linked fibrin. DIC is due to infections (e.g. HIV[64]), cancer, obstetrical complications, connective tissue disorders.	Elevated D-dimer, normal or abnormal PT, aPTT and fibrinogen	Treat underlying condition. Plasma and platelet replacement
Recent cardiovascular procedures, prosthetic heart valve	Cardiac catheterization, angioplasty, vascular bypass[24]	NS	NS
Severe hypertension	Accelerated blood pressure greater than 200/120 mmHg[24]	Normal[16]	Treat hypertension
Cancer/chemotherapy	Cumulative, dose-dependent drug toxicity of mitomycin and gemcitabine; insidious onset; possibly develop chronic renal failure	Normal or subnormal[16,65]	Stop inciting drug. Plasma exchange or immunosuppressive uncertain

aPTT = activated partial thromobplastin time; **DIC** = disseminated intravascular coagulation; **FFP** = fresh frozen plasma; **HELLP** = Haemolytic anaemia, Elevated Liver enzymes and Low Platelet count; **NS** = not stated; **PT** = prothrombin time; **SLE** = systemic lupus erythematosus; **TTP** = thrombotic thrombocytopenic purpura; **vWF** = von Willebrand factor.

Table II. Classification of transplantation-associated thrombotic microangiopathy

Treatment-related risk factors	Examples
Conditioning regimen	1. Total body irradiation[37,66,67] 2. High-dose busulfan,[68] fludarabine,[6,69] pentostatin[25]
Donor type	Matched unrelated donor[10,14,66,67,70-73]
Immunosuppression	1. Calcineurin inhibitors (ciclosporin,[36,74-78] tacrolimus[68,75,79]). Ciclosporin induces detachment of endothelial cells, initiating intrinsic coagulation system[78] 2. mTOR inhibitors (sirolimus)[75,80,81]
Infection	Iatrogenic infections: bacterial, fungal, viral (CMV, HHV-6)[82] – lipopolysaccharides and activation of host cells[75]
Transplantation	1. Acute graft-versus-host disease[36,10,70,66,73] 2. ABO incompatibility[6]

CMV = cytomegalovirus; **HHV-6** = human herpesvirus-6; **mTOR** = mammalian target of rapamycin.

should always be in the differential diagnosis because these medical and haematological conditions could also be present.

4. Epidemiology and Risk Factors

The true incidence of TA-TMA is not known because the enormous range reflects the different diagnostic criteria used. George et al.[5] published a review of 35 articles including 447 patients with TA-TMA and identified 28 different sets of diagnostic criteria. Furthermore, of 35 autopsy reports from the 447 patients, 11 explicitly reported no evidence of TA-TMA and none of the 35 reported systemic microthrombosis. These findings underscore the challenge of accurately diagnosing TA-TMA in HSCT recipients. TA-TMA can occur within the first few weeks following HSCT or can occur as a late complication because this event has been reported to occur up to 8 months after HSCT.[9]

Demographically, female sex, Black race, advanced primary disease, prior medical history of severe hepatic dysfunction and older age are associated with a higher risk of developing TA-TMA.[10,16,34,66,70,71] Treatment-related risk factors for developing TA-TMA include use of unrelated donor transplants ($p = 0.046$),[10,14,66,67,70,72,73] fludarabine-based nonmyeloablative conditioning transplants,[14,83] use of HLA-mismatched donors,[70] and administration of myeloablative conditioning with high-dose busulfan (16 mg/kg) and total body irradiation.[37,66,67] Anti-inflammatory agents used in HSCT are also associated with development of TA-TMA. These agents include calcineurin inhibitors such as ciclosporin,[36,74-76] tacrolimus[68,75,84] (both prevent the production of IL-2), and sirolimus,[75,80,81] which decreases cellular proliferation by inhibiting G1-S phase cell division. Finally, HSCT recipients who develop infection or GVHD have a higher risk of TA-TMA.[35,36,66,70,73] There were no differences in the incidence of TA-TMA according to graft source (i.e. BMT vs PBSCT).[10] The wide prevalence of TA-TMA in HSCT recipients may reflect the interaction of numerous factors, including the possibility that some patients with atypical HUS syndromes reflecting changes in complement protein regulators (e.g. Factors H and I) could also contribute.[85]

5. Prognosis

In patients with TA-TMA, poor prognostic indicators include the following: (i) aged >18 years; (ii) having a graft source from an unrelated or haploidentical donor;[66] (iii) at least five schistocytes per high-power field on peripheral smear;[86] (iv) TA-TMA in the absence of sirolimus exposure;[80] and (v) nephropathy.[10] Even with use of reduced-intensity conditioning, the incidence of TA-TMA may be as high as 10%; risk factors in this setting include the development of acute GVHD ($p = 0.007$) and ABO incompatibility ($p = 0.03$).[6]

On the other hand, higher response rates have been seen in the absence of nephropathy.[10] Kersting et al.[76] noted that patients who had severe acute renal failure (49.6% of allogeneic myeloablative stem cell transplantated patients) had poor outcome with an increased risk of developing TA-TMA. The association of these risk factors and poor prognostic indicators with TA-TMA again may demonstrate the shared pathophysiological mechanism of endothelial damage in TMA.

6. Treatment

Treatment of TA-TMA is based on the underlying aetiology, especially if ascertained. In general, therapy usually includes the following: (i) eliminating any risk factors for TA-TMA; and (ii) using medications such as daclizumab, defibrotide and rituximab. The mechanisms whereby these agents may be efficacious are uncertain because the proposed aetiology of TA-TMA in the HSCT recipient remains clouded. Plasma exchange and plasma infusion do not appear to have a role in this setting (as discussed in section 6.1).

6.1 Eliminating Risk Factors and Consideration of Plasma Exchange

Calcineurin inhibition has been linked to the development of TMA. The initial treatment approach should be replacement of ciclosporin or tacrolimus with alternative immunosuppressive medications. Withdrawal of ciclosporin along with initiation of plasma exchange has shown a response rate of 63%.[6] In idiopathic TTP, plasma exchange removes any autoantibody inhibiting ADAMTS-13 activity and repletes ADAMTS-13 in the fresh frozen plasma infusion. Because TA-TMA is independent of ADAMTS-13 activity, institution of plasma exchange benefits only a few patients.[87-89] Response rates after plasma exchange procedures are 0–49%[12,89] compared with 78–91% in patients with idiopathic TTP.[90,91] Although in one study, the mortality rate directly attributable to plasma exchange performed in patients suspected of having idiopathic TTP and HUS was only 2.4%, the morbidity rate was high at 28%.[79]

Plasma exchange procedures are associated with a number of complications including systemic infection, thrombosis, haemorrhage, pneumothorax, pericardial tamponade, hypoxia, hypotension, serum sickness and anaphylaxis associated with plasma infusion. Furthermore, although platelet transfusion is not usually recommended in idiopathic TTP patients except those with life-threatening haemorrhage, patients with TA-TMA may need platelet transfusion as a result of defective platelet production from their transplant.[89] This fact may contribute to the disease process.

Given the uncertainty of effectiveness of plasma exchange and the high complication rate in this highly immunocompromised patient population, we do not advocate use of this procedure because of the unfavourable benefit : risk ratio.

6.2 Daclizumab

Daclizumab is a humanized monoclonal anti-CD25 antibody, which targets the α chain of the IL-2 receptor.[92] First approved by the US FDA in 1997, historically, daclizumab has been used to decrease the incidence of acute rejection in solid organ transplants including cardiac,[77,93] liver, renal[94-96] and lung transplantation.[77,95] Daclizumab therapy has also been used in T-cell-mediated autoimmune disorders such as non-infectious uveitis,[97] multiple sclerosis,[98-100] pure red cell aplasia[101] and aplastic anaema.[102] In HSCT, daclizumab has been used to prevent or treat acute GVHD.[103,104] In one study, prophylactic use of daclizumab has been shown to improve survival ($p < 0.001$),[103] but in another study, this agent was deemed ineffective as treatment for steroid-refractory acute GVHD.[104] The mechanism by which daclizumab is effective in these disorders is thought to be depletion of alloreactive T cells.[104] Daclizumab recognizes and binds to the α subunit of the IL-2 receptor on the activated alloantigen-reactive T cells, thereby depleting production of IL-2.[105-107]

Several studies demonstrated improvement in TA-TMA patients by substituting daclizumab for a calcinuerin inhibitor. Daclizumab does not have a direct cytotoxic effect on lymphocytes and endothelial cells[78] or have nephrotoxic potential. Wolff et al.[75] substituted daclizumab using a loading dose of 2 mg/kg and then 1 mg/kg intravenously per week. Significant improvements were noted in 11 of 13 TA-TMA patients (9 of 13 with complete response, 2 of 13 had stable disease)[75] [table III]. This success may be attributed to daclizumab not possessing the potential for endothelial damage seen with other medications. This small study does not represent the 'standard' transplant population as many patients received

Table III. Daclizumab for the treatment of transplantation-associated thrombotic microangiopathy (TMA) [reproduced from Wolff et al.,[75] with permission from Macmillan Publishers Ltd, copyright 2006]

Diagnosis and status	Median age (y)	HSCT type	Conditioning regimen	GVHD prophylaxis	Serum LDH at TMA (U/L)	GVHD therapy	TMA therapy with daclizumab	Response
T-ALL NR	23	MUD	TBI, Cy, VP16, Camp, Treo, Flud, ATG	CsA	6686	FK, daclizumab etanercept, MP, sirolimus	MP, sirolimus	NR
AML CR1	54	MRD	Treo, Flud, ATG	CsA, MMF	1116	CsA, MP, daclizumab etanercept	MP	CR
MM Rel 1	41	MUD	Mel, Flud, ATG	CsA, MTX	2025	CsA, MP, ATG, P	MP, pentostatin	NR
HD PR2	18	MUD	Treo, Cy	CsA, MTX	308	CsA, MP	P	CR
AML CR2	26	MUD	TBI, AraC	CsA, MMF	2112	CsA, MP	Etanercept, MP	CR
MM CR1	33	MUD-BM	Treo, Flud, ATG	CsA, MTX	614	CsA, MP, etanercept, daclizumab	MP	CR
CML AP1	53	MUD	Treo, Flud, ATG	CsA, MTX	429	CsA, MP	MP, pentostatin	CR
MM Rel 1	50	MUD	Treo, Flud, ATG	CsA, MTX	824	CsA, MP etanercept, daclizumab	MP, pentostatin, etanercept	NC
CML CP	44	MUD	Treo, Flud, ATG	CsA, MTX	1244	CsA, MP, sirolimus, pentostatin, daclizumab, etanercept, MMF	MP	NC
CML CP	48	MRD	TBI, Arac	CsA, MMF	908	FK, P, sirolimus	P	CR
AML CR2	69	MUD	TBI, Flud	CsA, MMF	697	CsA, MP	MP	CR
AML CR1	48	MRD	Flud, BCNU, Mel	CsA, MMF	366	CsA, P, MMF	P, MMF, budesonide	CR
AML CR3	31	MMUD	Treo, Flud	CsA, MMF	69	CsA, MP, MMF	MP, MMF, FK (resumed therapy on day 89 after transplant)	CR

AML = acute myeloid leukaemia; **AraC** = cytarabine; **ATG** = antithymocyte globulin; **BCNU** = carmustine; **Camp** = Campath; **CML AP1** = chronic myeloid leukaemia, first accelerated phase; **CML CP** = chronic myeloid leukaemia, chronic phase; **CR (1, 2, 3)** = complete response (first, second, third); **CsA** = ciclosporin; **Cy** = cyclophosphamide; **FK** = tacrolimus; **Flud** = fludarabine; **GVHD** = graft-versus-host disease; **HD PR2** = Hodgkin lymphoma, second partial remission; **HSCT** = haematopoietic stem cell transplantation; **LDH** = lactate dehydrogenase; **Mel** = melphalan; **MM** = multiple myeloma; **MMF** = mycophenolate mofetil; **MMUD** = mismatched unrelated donor; **MP** = methylprednisolone; **MRD** = matched related donor; **MTX** = methotrexate; **MUD** = matched unrelated donor; **MUD-BM** = matched unrelated donor-bone marrow stem cell graft; **NC** = no change; **NR** = no response; **P** = prednisolone/prednisone; **Rel 1** = first relapse; **T-ALL** = T-cell acute lymphoblastic leukaemia; **TBI** = total body irradiation; **Treo** = treosulfan; **VP16** = etoposide.

treosulfan and fludarabine conditioning, and it may be hard to generalise upon this favourable result.

The serum half-life of daclizumab is 20 days.[105] Adverse effects include rash,[101] an infection diathesis for severe bacterial infections, systemic candidiasis and aspergillosis as well as cytomegalovirus reactivation.[75] In addition, concomitant cytolytics should be avoided because of a higher risk of infection-related mortality.[93] Furthermore, because inhibition of the IL-2 receptor results in inhibition of the graft-versus-leukaemia effect, there may be potential for an increase in leukaemia recurrence.[103,108]

6.3. Defibrotide

Defibrotide, a polydeoxyribonucleotide salt, has been shown to protect against endothelial

damage[109] by inhibiting TNFα-mediated endothelial cell apoptosis *in vitro*[110] and has also demonstrated profibrinolytic, antithrombotic,[111] anti-inflammatory and thrombolytic[112,113] activity. Specifically, defibrotide reduces proinflammatory tissue factor expression by endothelial cells and decreases the activity of PAI-1 and increases endogenous tissue plasminogen activator (t-PA) function *in vitro*.[114] Defibrotide is used in the non-HSCT setting for recurrent TTP.[115]

In some studies, hepatic VOD that develops after HSCT approaches 20%, with mortality ranging from 7–50%.[116] Defibrotide therapy has improved patient outcomes for VOD that develops after HSCT (30–60% complete response rate)[53,54,112,117-121] as well as VOD caused by gemtuzumab ozogamicin treatment.[122] Furthermore, defibrotide reduces the incidence of VOD when used as prophylaxis.[123,124] VOD, caused by sinusoidal endothelial cell damage initiated by Factor VIII and fibrinogen deposition within the sinusoidal and venular wall,[116] would be predicted as an excellent target for this therapy.

As a result of these potential benefits, defibrotide therapy has been attempted and used successfully for TA-TMA. Corti et al.[125] reported that 5 of 12 affected patients given oral defibrotide 40 mg/kg/day achieved complete remission, while three patients achieved partial remission. Wolff et al.[75] used a combination of both defibrotide and daclizumab, and Besisik et al.[118] used defibrotide with plasma exchange in a patient who subsequently achieved complete remission (table IV). Defibrotide therapy was generally well tolerated but a single case report of anaphylactic shock[126] has been published.

Table IV. Defibrotide for the treatment of transplantation-associated thrombotic microangiopathy

Study	Diagnosis	Age (y)	HSCT type	Serum LDH (U/L)	Additional therapy to defibrotide	Defibrotide dose (mg/kg/day)	Response
Wolff et al.[75]	CML AP1	53	MUD	429	MP, pentostatin, daclizumab	20 IV	CR
	MM Rel 1	50	MUD	824	MP, pentostatin, etanercept, daclizumab	20 IV	NC
	AML CR3	31	MMUD	525	MP, MMF, FK (resumed therapy on day 89 after transplant), daclizumab	20 IV	CR
Besisik et al.[118]	Lymphoblastic lymphoma	19	MRD		Plasma exchange		CR
Corti et al.[125]	AML	3	MRD	2×ULN		40 PO	CR
	FEL	1	MUD	3×ULN		40 PO	CR
	ALL	12	MRD	2×ULN		40 PO	CR
	ALL	7	MUD	5×ULN		40 PO	CR
	CML	35	MUD	7×ULN	Plasma exchange	40 PO	NR
	ALL	16	MUD	2×ULN	Plasma exchange	40 PO	PR
	HL	33	MRD	2×ULN		40 PO	CR
	ALL	20	MUD	2×ULN	Plasma exchange	40 PO	NR
	ALL	35	MUD	5×ULN		40 PO	PR
	AML	37	MRD	2×ULN		40 PO	CR
	CML	55	MRD	1×ULN	Plasma exchange	40 PO	NR
	AML	30	MUD	2×ULN	Plasma exchange	40 PO	PR

ALL = acute lymphoblastic leukaemia; **AML** = acute myeloid leukaemia; **AML CR3** = acute myeloid leukaemia, third complete remission; **CML** = chronic myeloid leukaemia; **CML AP1** = chronic myeloid leukaemia, first accelerated phase; **CR** = complete response; **FEL** = familial lymphohistiocytosis; **FK** = tacrolimus; **HSCT** = haematopoietic stem cell transplantation; **HL** = Hodgkin's lymphoma; **IV** = intravenous; **LDH** = lactate dehydrogenase; **MMF** = mycophenolate mofetil; **MM Rel 1** = multiple myeloma, first relapse; **MP** = methylprednisolone; **MMUD** = mismatched unrelated donor; **MRD** = matched related donor; **MUD** = matched unrelated donor; **NC** = no change; **NR** = no response; **PR** = partial response; **PO** = oral; **ULN** = upper limit of normal.

6.4. Rituximab

Rituximab, an anti-CD20 antibody, has been used to treat various types of non-TA-TMA disorders including idiopathic TTP,[127-129] immune thrombocytopenic purpura, other haematological disorders (e.g. drug-induced thrombocytopenia/anaemia and autoimmune/alloimmune haemolytic anaemia) and non-haematological immune-mediated disorders (e.g. systemic lupus erythematosus, rheumatoid arthritis and antiphospholipid antibody syndrome).[130] Rituximab may be useful to eliminate the antibodies to ADAMTS-13 in TTP.[127,128,131]

Au et al.[132] reported rituximab-induced complete remission in four of five patients with TA-TMA that was refractory to plasma exchange and prednisone therapy. All patients had low blood concentrations of ADAMTS-13 and one patient had an anti-ADAMTS-13 antibody. Concentration of ADAMTS-13 as well as the anti-ADAMTS-13 antibody remained stable despite the rituximab-induced remission. It is unclear whether these patients who were diagnosed as having TA-TMA, may actually have had TTP, clouding the role of rituximab.

Ostronoff and colleagues[133] reported a single BMT patient with HUS who was treated successfully with rituxumab 375 mg/m^2 weekly for 4 weeks as indicated by improvement in haemoglobin, creatinine, serum LDH and bilirubin after 1 week (table V). Unlike non-TA-TMA conditions, in which the need for further treatment after the initial four standard infusions of rituximab is guided by the ADAMTS-13 activity and continuing presence of IgG antibodies against ADAMTS-13,[128] there are no guidelines for the length of therapy for TA-TMA; ADAMTS-13 levels are irrelevant and do not increase with successful treatment.[132] Therefore, the length of treatment should be based on clinical improvement. Adverse events associated with rituximab reported in the literature in other settings include hypotension,[131] allergic reactions and progressive multifocal leukoencephalopathy.[136]

7. Prevention

Medications such as rituximab and daclizumab, which have been effectively used to treat TA-TMA, have also been used as prophylaxis to decrease the risk for development of TA-TMA.

Table V. Rituximab for the treatment of transplantation-associated thrombotic microangiopathy

Study	Diagnosis	HSCT conditioning	Duration of plasma exchange (d)	Other therapy	Response	Rituximab regimen
Gallerani et al.[134]	MM	VAD then Auto	9	P	Remission	Resumed therapy on day 69 after transplant; 600 mg/m^2 weekly for 4 wk
Au et al.[132]	ALL	MUD/Cy-TBI	10	P, V, IVIg	Remission	375 mg/m^2 weekly for 4 wk
	WAS	MUD/BuCy-ATG	14	P, MMF	Remission	
	NHL	MUD/Cy-TBI	13	P, MMF	Remission	
	CML	MUD/Cy-TBI	17	P	Remission	
	AML	MUD/BuCy	7	P	No remission	
Au et al.[135]	ALL	MUD/Cy-TBI	0	P, MMF, ATG,	Remission	375 mg/m^2 weekly for 4 wk
	CML-BT	MUD/BuCy	0	P	No remission[a]	375 mg/m^2 weekly for 2 wk
Ostonoff et al.[133]	ALL	MRD/Cy-TBI	6	None	Remission	Began therapy on day 17 after transplant; 375 mg/m^2 weekly for 4 wk

a Patient died with fungal sepsis after two courses of rituximab.

ALL = acute lymphocytic leukaemia; **AML** = acute myeloid leukaemia; **ATG** = antithymocyte globulin; **Auto** = autologous stem cell transplantation; **Bu** = busulfan; **CML** = chronic myeloid leukaemia; **CML-BT** = chronic myeloid leukaemia in blastic transformation; **Cy** = cyclophosphamide; **HSCT** = haematopoietic stem cell transplantation; **IVIg** = intravenous immunoglobulin; **MM** = multiple myeloma; **MMF** = mycophenolate mofetil; **MRD** = matched related donor; **MUD** = matched unrelated donor; **NHL** = non-Hodgkin's lymphoma; **P** = prednisolone/prednisone; **TBI** = total body irradiation; **V** = vincristine; **VAD** = vincristine, doxorubicin, dexamethasone; **WAS** = Wiskott-Aldrich Syndrome.

Rituximab was given to prevent TTP in an asymptomatic patient with low ADAMTS-13 activity after three prior occurrences that were successfully treated with plasma exchange.[137] Rituximab as part of a conditioning regimen appears to decrease the incidence of acute GVHD.[138] Limited success in treating acute and chronic GVHD has been shown with daclizumab 1 mg/kg for 5 days and then once every week until day 28.[108] By reducing likelihood of acute GVHD, a risk factor for development of TA-TMA, there is potential for decreasing the incidence of TA-TMA.

Takatsuka and colleagues[139] used eicosapentaenoic acid (EPA), a selective inhibitor of DNA polymerase,[140] to prevent allogeneic HSCT associated TA-TMA in 16 consecutive patients. Seven received EPA beginning 3 weeks prior to unrelated donor allogeneic transplant and continued until 180 days after transplantation, while nine patients did not receive EPA. TA-TMA developed in four untreated patients but in none of the treated patients. Systemic inflammatory cytokine levels, including leukotriene B4, thromboxane A_2 and PGI_2, were significantly lower in treated patients, as were TNFα, IFNγ, IL-10 and surrogates for endothelial damage, including thrombomodulin and PAI-1.[139]

8. Recommendation and Future Directions

Endothelial injury is common after allogeneic HSCT and results from exposure to conditioning regimens, calcineurin inhibitors, GVHD and opportunistic infections. These factors simultaneously contribute to the development of TA-TMA. Monitoring production of endothelial microparticles or protein concentration changes of vWF, thrombomodulin and PAI-1, which occur in the setting of endothelial injury, may be useful in detecting early onset of TA-TMA. Early detection may provide opportunities to identify reversible causes of endothelial injury, prevent development of overt TA-TMA and improve patient outcomes. Treatment consists of substituting calcinuerin inhibitors with daclizumab, especially in those with mild to moderate TA-TMA. Rituximab therapy or the addition of defibrotide may also be beneficial. Plasma exchange can be considered in select patients but, in general, is not recommended.

On-going studies to identify other drugs that may be useful in the prevention or treatment of TA-TMA involve medications that reduce endothelial inflammatory response. Candidate agents include HMG-CoA reductase inhibitors,[141,142] iloprost, a prostacyclin analogue, which reduces plasma markers of endothelial cell activation and injury in patients with rheumatoid arthritis,[143] and bosentan, an endothelin receptor antagonist, which protects against ischaemia/reperfusion-induced endothelial injury *in vivo*.[144] Antithrombin III, which prevents against endothelial damage, has been used successfully to treat TA-TMA in a case report of two patients.[145] In solid organ transplantation, IgG has also been used successfully for the treatment of TA-TMA in two patients.[146] These results merit further investigation of these possible treatment strategies.

TA-TMA is a heterogeneous, devastating event occurring as a result of a combination of idiopathic treatment-related endothelial damage and the underlying disease process. Further research is necessary to determine the mechanism(s) of endothelial inflammation in the HSCT setting. Although there are several case reports and small trials providing optimistic treatment options for TA-TMA, these findings need to be corroborated in larger prospective trials.

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References

1. National Marrow Donor Program®. Facts and figures [online]. Available from URL: http://www.marrow.org/NEWS/MEDIA/Facts_and_Figures/facts_figures.pdf [Accessed 2008 Nov 13]

2. Burt RK, Loh Y, Pearce W, et al. Clinical applications of blood-derived and marrow-derived stem cells for non-malignant diseases. JAMA 2008; 299: 925-36
3. Bensinger WI, Martin PJ, Storer B, et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. N Eng J Med 2001; 344: 175-81
4. Batts E, Lazarus HM. Diagnosis and treatment of transplantation-associated thrombotic microangiopathy: real progress or are we still waiting? Bone Marrow Transplant 2007; 40: 709-19
5. George JN, Li X, McMinn JR, et al. Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome following allogeneic hematopoietic stem cell transplantation: a diagnostic dilemma. Transfusion 2004; 44: 294-304
6. Worel N, Greinix HT, Leitner G, et al. ABO-incompatible allogeneic hematopoietic stem cell transplantation following reduced-intensity conditioning: close association with transplant-associated microangiopathy. Transfus Apher Sci 2007; 36: 297-304
7. Pettitt AR, Clark RE. Thrombotic microangiopathy following bone marrow transplantation. Bone Marrow Transplant 1994; 14: 495-504
8. Zeigler ZR, Shadduck RK, Nemunaitis J, et al. Bone marrow transplant-associated thrombotic microangiopathy: a case series. Bone Marrow Transplant 1995; 15: 247-53
9. Iacopino P, Pucci G, Arcese W, et al. Severe thrombotic microangiopathy: an infrequent complication of bone marrow transplantation. Gruppo Italiano Trapianto Midollo Osseo (GITMO). Bone Marrow Transplant 1999; 24: 47-51
10. Ruutu T, Hermans J, Niederwieser D, et al. Thrombotic thrombocytopenic purpura after allogeneic stem cell transplantation: a survey of the European Group for Blood and Marrow Transplantation (EBMT). Br J Haematol 2002; 118: 1112-9
11. Fuge R, Bird JM, Fraser A, et al. The clinical features, risk factors and outcome of thrombotic thrombocytopenic purpura occurring after bone marrow transplantation. Br J Haematol 2001; 113: 58-64
12. Daly AS, Xenocostas A, Lipton JH. Transplantation-associated thrombotic microangiopathy: twenty-two years later. Bone Marrow Transplant 2002; 30: 709-15
13. Ho VT, Cutler C, Carter S, et al. Blood and Marrow Transplant Clinical Trials Network Toxicity Committee consensus summary: thrombotic microangiopathy after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2005; 11: 571-5
14. Elliott MA, Nichols WL, Plumhoff EA, et al. Post-transplantation thrombotic thrombocytopenic purpura: a single-center experience and a contemporary review. Mayo Clin Proc 2003; 78: 421-30
15. Sadler JE. Thrombotic thrombocytopenic purpura: a moving target. Hematol Am Soc Hematol Educ Program 2006: 415-20
16. Terrell DR, Williams LA, Vesely SK. The incidence of thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: all patients, idiopathic patients, and patients with severe ADAMTS-13 deficiency. J Thromb Haemost 2005; 3: 1432-6
17. Huang J, Roth R, Heuser JE, et al. Integrin $\alpha v\beta 3$ on human endothelial cells bind von Willebrand factor strings under fluid shear stress. Blood 2009; 113 (7): 1589-97. Epub 2008 Oct 16
18. Rock G, Shumak KH, Sutton DM, et al. Cryosupernatant as replacement fluid for plasma exchange in thrombotic thrombocytopenic purpura. Members of the Canadian Apheresis Group. Br J Haematol 1996; 94: 383-6
19. Furlan M, Robles R, Solenthaler M, et al. Deficient activity of von Willebrand factor-cleaving protease in chronic relapsing thrombotic thrombocytopenic purpura. Blood 1997; 89: 3097-103
20. Feyes HB, Liu F, Dong N, et al. ADAMTS-13 plasma level determination uncover antigen absence in acquired thrombotic thrombocytopenic purpura and ethnic differences. J Thromb Haemost 2006; 4: 955-62
21. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. N Engl J Med 1998; 339: 1585-94
22. Furlan M, Robles R, Galbusera M, et al. Von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. N Engl J Med 1998; 339: 1578-84
23. Manea M, Kristoffersson AC, Tsai HM, et al. ADAMTS13 phenotype in plasma from normal individuals and patients with thrombotic thrombocytopenic purpura. Eur J Pediatr 2007; 166: 249-57
24. Tsai HM. Advances in the pathogenesis, diagnosis, and treatment of thrombotic thrombocytopenic purpura. J Am Soc Nephrol 2003; 14: 1072-81
25. Tsai HM. Is severe deficiency of ADAMTS-13 specific for thrombotic thrombocytopenic purpura? Yes. J Thromb Haemost 2003; 1: 625-31
26. Lammle B, Kremer Hovinga JA, Alberio L. Thrombotic thrombocytopenic purpura. J Thromb Haemost 2005; 3: 1663-75
27. Jimenez JJ, Jy W, Mauro LM, et al. Elevated endothelial microparticles in thrombotic thrombocytopenic purpura: findings from brain and renal microvascular cell culture and patients with active disease. Br J Haematol 2001; 112: 81-90
28. Eremina V, Jefferson JA, Kowalewska J, et al. VEGF inhibition and renal thrombotic microangiopathy. N Engl J Med 2008; 358: 1129-36
29. Mitra D, Jaffe E, Weksler B, et al. Thrombotic thrombocytopenic purpura and sporadic hemolytic-uremic syndrome plasmas induced apoptosis in restricted lineages of human microvascular endothelial cells. Blood 1997; 89: 1224-34
30. Starke R, Machin S, Scully M, et al. The clinical utility of ADAMTS13 activity, antigen, and autoantibody assays in thrombotic thrombocytopenic purpura. Br J Haematol 2007; 136: 649-55
31. Bohm M, Betz C, Miesbach W, et al. The course of ADAMTS-13 activity and inhibitor titre in the treatment of thrombotic thrombocytopenic purpura with plasma exchange and vincristine. Br J Haematol 2005; 129: 644-52
32. van der Plas RM, Schiphorst ME, Huizinga EG, et al. von Willebrand factor proteolysis is deficient in classic, but not in bone marrow transplantation-associated, thrombotic thrombocytopenic purpura. Blood 1999; 93: 3798-802
33. Kentouche K, Zintl F, Angerhaus D, et al. Von Willebrand factor-cleaving protease (ADAMTS13) in the course of

stem cell transplantation. Semin Thromb Hemost 2006; 32: 98-104

34. Peyvandi F, Siboni SM, Lambertenghi Deliliers D, et al. Prospective study on the behaviour of the metalloprotease ADAMTS13 and of von Willebrand factor after bone marrow transplantation. Br J Haematol 2006; 134: 187-95
35. Fuge R, Bird I, Fraser A, et al. The clinical features, risk factors and outcome of thrombotic thrombocytopenic purpura occurring after bone marrow transplantation. Br J Haematol 2001; 113: 58-64
36. Holler E, Kolb HJ, Hiller E, et al. Microangiopathy in patients on cyclosporine prophylaxis who developed acute graft versus-host disease after HLA-identical bone marrow transplantation. Blood 1989; 73: 2018-24
37. Chappell ME, Keeling DM, Prentice HG. Haemolytic-uraemic syndrome after bone marrow transplantation: an adverse effect of total body irradiation? Bone Marrow Transplant 1988; 3: 339-47
38. Dang CT, Magid M, Weksler B, et al. Enhanced endothelial apoptosis in splenic tissue of patients with thrombotic thrombocytopenic purpura. Blood 1999; 93: 1264-70
39. Goon PK, Lip GY, Boos CJ, et al. Circulating endothelial cells, endothelial progenitor cells, and endothelial microparticles in cancer. Neoplasia 2006; 8: 79-88
40. Jimenez JJ, Jy W, Mauro LM, et al. Endothelial microparticles released in thrombotic thrombocytopaenic purpura express von Willebrand factor and markers of endothelial activation. Br J Haematol 2003; 123: 896-902
41. Morel O, Toti F, Hugel B, et al. Procoagulant microparticles: disrupting the vascular homeostasis equation? Arterioscl Thromb Vasc Biol 2006; 26: 2594-604
42. Mallat Z, Benamer H, Hugel B, et al. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. Circulation 2000; 101: 841-3
43. Jy W, Jimenz J, Mauro LM, et al. Endothelial microparticles induce formation of platelet aggregates via a von Willebrand factor/ristocetin dependent pathway, rendering them resistant to dissociation. J Thromb Haemost 2005; 3: 1301-8
44. Pihusch V, Rank A, Steber R, et al. Endothelial cell-derived microparticles in allogeneic hematopoietic stem cell recipients. Transplantation 2006; 81: 1405-9
45. Nomura S, Ishii K, Kanazawa S, et al. Significance of elevation in cell-derived microparticles after allogeneic stem cell transplantation: transient elevation of platelet-derived microparticles in TMA/TTP. Bone Marrow Transplant 2005; 36: 921-2
46. Cohen H, Bull HA, Seddon A, et al. Vascular endothelial cell function and ultrastructure in thrombotic microangiopathy following allogeneic bone marrow transplantation. Eur J Hematol 1989; 43: 207-14
47. Takatsuka H, Wakae T, Mori A, et al. Thrombotic thrombocytopenic purpura and hemolytic uremic syndrome following allogeneic bone marrow transplantation. Bone Marrow Transplant 2002; 29: 907-11
48. Testa S, Manna A, Porcellini A, et al. Increased plasma level of vascular endothelial glycoprotein thrombomodulin as an early indicator of endothelial damage in bone marrow transplantation. Bone Marrow Transplant 1996; 18: 383-8
49. Salat C, Holler E, Kolb H-J, et al. Endothelial cell markers in bone marrow transplant recipients with and without acute graft versus host disease. Bone Marrow Transplant 1997; 19: 909-14
50. Nurnberger W, Michelmann I, Burdach S, et al. Endothelial dysfunction after bone marrow transplantation: increase of soluble thrombomodulin and PAI-1 in patients with multiple transplant-related complications. Ann Hematol 1998; 76: 61-5
51. Schmaier AH, Srikanth S, Elghetany MT, et al. Hemostatic/fibrinolytic protein changes in C3H/HeN mice infected with *Rickettsia conorii*: a model for rocky mountain spotted fever. Thromb Haemost 2001; 86: 871-9
52. Qu L, Kiss JE. Thrombotic microangiopathy in transplantation and malignancy. Semin Thromb Hemost 2005; 31: 691-9
53. Richardson PG, Murakami C, Jin Z, et al. Multi-institutional use of defibrotide in 88 patients after stem cell transplantation with severe veno-occlusive disease and multisystem organ failure: response without significant toxicity in a high-risk population and factors predictive of outcome. Blood 2002; 100: 4337-43
54. Corbacioglu S, Greil J, Peters C, et al. Defibrotide in the treatment of children with veno-occlusive disease (VOD): a retrospective multicentre study demonstrates therapeutic efficacy upon early intervention. Bone Marrow Transplant 2004; 33: 189-95
55. Ruutu T, Giovanni B, 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
56. Arai S, Allan C, Streiff M, et al. von Willebrand factor-cleaving protease activity and proteolysis of von Willebrand factor in bone marrow transplant-associated thrombotic microangiopathy. Hematol J 2001; 2: 292-9
57. Kersting S, Verdonck LF. Stem cell transplantation nephropathy: a report of six cases. Biol Blood Marrow Transplant 2007; 13: 638-43
58. George JN. Clinical practice: thrombotic thrombocytopenic purpura. N Engl J Med 2006; 354: 1927-35
59. Studt JD, Hovinga JA, Radonic R, et al. Familial acquired thrombotic thrombocytopenic purpura: ADAMTS13 inhibitory autoantibodies in identical twins. Blood 2004; 103: 4195-7
60. Chandler WL, Jelacic S, Boster DR, et al. Prothrombotic coagulation abnormalities preceding the hemolytic-uremic syndrome. N Engl J Med 2002; 346: 23-32
61. Tsai HM, Chandler WL, Sarode R, et al. von Willebrand factor and von Willebrand factor -cleaving metalloprotease activity in *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. Pediatr Res 2001; 49: 653-9
62. Bennett CL, Kim B, Zakarija A, et al. Two mechanistic pathways for thienopyridine-associated thrombotic thrombocytopenic purpura: a report from the SERF-TTP Research Group and the RADAR Project. J Am Coll Cardiol 2007; 50: 1138-43
63. Tsai HM, Rice L, Sarode R, et al. Antibody inhibitors to von Willebrand factor metalloproteinase and increased binding of von Willebrand factor to platelets in ticlopidine-associated thrombotic thrombocytopenic purpura. Ann Intern Med 2000; 132: 794-9

64. Laurence J, Mitra D, Steiner M, et al. Plasma from patients with idiopathic and human immunodeficiency virus-associated thrombotic thrombocytopenic purpura induces apoptosis in microvascular endothelial. Blood 1996; 87: 3245-54
65. Fontana S, Gerritsen HE, Kremer-Hovinga J, et al. Microangiopathic haemolytic anaemia in metastasizing malignant tumours is not associated with a severe deficiency of the von Willebrand factor-cleaving protease. Br J Haematol 2001; 1: 100-2
66. Uderzo C, Bonanomi S, Alessandro B, et al. Risk factors and severe outcome in thrombotic microangiopathy after allogeneic hematopoietic stem cell transplantation. Transplantation 2006; 82: 638-44
67. Paquette RL, Tran L, Landaw EM. Thrombotic microangiopathy following allogeneic bone marrow transplantation is associated with intensive graft-versus-host disease prophylaxis. Bone Marrow Transplant 1998; 22: 351-7
68. Nakamae H, Yamane T, Hasegawa T, et al. Risk factor analysis for thrombotic microangiopathy after reduced-intensity or myeloablative allogeneic hematopoietic stem cell transplantation. Am J Hematol 2006; 81: 525-31
69. Kalhs P, Brugger S, Schwarzinger I, et al. Association with cyclosporine and methylprednisone for graft versus-host disease prophylaxis. Transplantation 1995; 60: 949-57
70. Roy V, Rizvi MA, Vesely SK, et al. Thrombotic thrombocytopenic purpura-like syndromes following bone marrow transplantation: an analysis of associated conditions and clinical outcomes. Bone Marrow Transplant 2001: 27: 641-6
71. Takatsuka H, Nakajima T, Nomura K, et al. Change in clotting factors (7, 9 and 10) and hepatocyte growth factor in patients with thrombotic microangiopathy after bone marrow transplantation. Clin Transplant 2006; 20: 640-3
72. Uderzo C, Fumagalli M, De Lorenzo P, et al. Impact of thrombotic thrombocytopenic purpura on leukemic children undergoing bone marrow transplantation. Bone Marrow Transplant 2000; 26: 1005-9
73. Daly AS, Hasegawa WS, Upton JH, et al. Transplantation-associated thrombotic microangiopathy is associated with transplantation from unrelated donors, acute graft-versus-host disease and venoocclusive disease of the liver. Transfus Apher Sci 2002; 27: 3-12
74. Shulman H, Striker G, Deeg HJ, et al. Nephrotoxicity of cyclosporine A after allogeneic marrow transplantation-glomerular thromboses and tubular injury. N Engl J Med 1981; 305: 1392-5
75. Wolff D, Wilhelm S, Hahn J, et al. Replacement of calcineurin inhibitors with daclizumab in patients with transplantation-associated microangiopathy or renal insufficiency associated with graft-versus-host disease. Bone Marrow Transplant 2006; 38: 445-51
76. Kersting S, Koomans HA, Hene RJ, et al. Acute renal failure after allogeneic myeloablative stem cell transplantation: retrospective analysis of incidence, risk factors, and survival. Bone Marrow Transplant 2007; 39: 359-65
77. Kobashigawa J, David K, Morris AH, et al. Daclizumab is associated with decreased rejection and no increased mortality in cardiac transplant patients receiving MMF, cyclosporine, and corticosteroids. Transplant Proc 2005; 37: 1333-9
78. Bombeli T, Muller M, Straub PW, et al. Cyclosporine-induced detachment of vascular endothelial cells initiates the intrinsic coagulation system in plasma and whole blood. J Lab Clin Med 1996; 127: 621-34
79. Howard MA, Williams LA, Terrell DR, et al. Complications of plasma exchange in patients treated for clinically suspected thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. Transfusion 2006; 46: 154-6
80. Cutler C, Henry NL, Magee C, et al. Sirolimus and thrombotic microangiopathy after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2005; 11: 551-7
81. Couriel DR, Saliba R, Escalon MP, et al. Sirolimus in combination with tacrolimus and corticosteroids for the treatment of resistant chronic graft-versus-host disease. Br J Haematol 2005; 130: 409-17
82. Takatsuka H, Wakae T, Mori A, et al. Endothelial damage caused by cytomegalovirus and human herpesvirus-6. Bone Marrow Transplant 2003; 31: 475-9
83. Shimoni A, Yeshurun M, Hardan I, et al. Thrombotic microangiopathy after allogeneic stem cell transplantation in the era of reduced-intensity conditioning: the incidence is not reduced. Biol Blood Marrow Transplant 2004; 19: 484-93
84. Sarkodee-Adoo C, Sotirescu D, Sensenbrenner L, et al. Thrombotic microangiopathy in blood and marrow transplant patients receiving tacrolimus or cyclosporine A. Transfusion 2003; 359: 2078-83
85. Kavanagh D, Goodship TH, Richards A. Atypical haemolytic uraemic syndrome. Br Med Bull 2006; 77-78: 5-22
86. Martinez MT, Bucher CH, Stussi G, et al. Transplant-associated microangiopathy (TAM) in recipients of allogeneic hematopoietic stem cell transplants. Bone Marrow Transplant 2005; 36: 993-1000
87. Kida Y, Ishii T, Ando M, et al. A case report of thrombotic thrombocytopenic purpura and severe acute renal failure post non-myeloablative allogeneic peripheral stem cell transplantation treated with plasma exchange and hemodialysis. Ther Apher Dial 2007; 11: 402-6
88. Llamas P, Romero R, Cabrera R, et al. Management of thrombotic microangiopathy following allogeneic transplantation: what is the role of plasma exchange? Bone Marrow Transplant 1997; 20: 305-6
89. Teruya J, Styler M, Verde S, et al. Questionable efficacy of plasma exchange for thrombotic thrombocytopenic purpura after bone marrow transplantation. J Clin Apher 2001; 16: 169-74
90. Rock G, Shumak KH, Buskard NA, et al. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. Canadian Apheresis Study Group. N Engl J Med 1991; 6: 393-7
91. Rock G, Shumak KH, Kelton J, et al. Thrombotic thrombocytopenic purpura: outcome in 24 patients with renal impairment treated with plasma exchange. Canadian Apheresis Study Group. Transfusion 1992; 32: 710-14
92. Goebel J, Stevens E, Forrest K, et al. Daclizumab (Zenapax) inhibits early interleukin-2 receptor signal transduction events. Transpl Immunol 2000; 8: 153-9
93. Hershberger RE, Starling RC, Eisen HJ, et al. Daclizumab to prevent rejection after cardiac transplantion. N Engl J Med 2005; 352: 2705-13
94. Wiseman LR, Faulds D. Daclizumab: a review of its use in the prevention of acute rejection in renal transplant recipients. Drugs 1999; 58: 1029-42

95. Vincenti F, Nashan B, Light S. Daclizumab: outcome of phase III trials and mechanism of action. Transplant Proc 1998: 30; 2155-8
96. Ekberg H, Backman L, Tufveson G, et al. Daclizumab prevents acute rejection and improve patient survival post transplantation: 1 year pooled analysis. Transpl Int 2000; 13: 151-9
97. Nussenblatt RB, Thompson DJ, Li Z, et al. Humanized anti-interleukin-2 (IL-2) receptor alpha therapy: long term results in uveitis patients and preliminary safety and activity data for establishing parameters for subcutaneous administration. J Autoimmun 2003; 21: 283-93
98. Martin R. Humanized anti-CD25 antibody treatment with daclizumab in multiple sclerosis. Neurodegener Dis 2008; 5: 23-6
99. Bielekova B, Richert N, Howard T, et al. Humanized anti-CD25 (daclizumab) inhibits disease activity in multiple sclerosis patients failing to respond to interferon beta. Proc Natl Acad Sci U S A 2004; 101: 8705-8
100. Rose JW, Burns JB, Bjorklund J, et al. Daclizumab phase II trial in relapsing and remitting multiple sclerosis: MRI and clinical results. Neurology 2007; 69: 785-9
101. Sloand EM, Scheinberg P, Maciejewski J, et al. Brief communication: successful treatment of pure red-cell aplasia with an anti-interleukin-2 receptor antibody (Daclizumab). Ann Intern Med 2006; 144: 181-5
102. Maciejewski JP, Sloand EM, Nunez O, et al. Recombinant humanized anti-IL-2 receptor antibody (daclizumab) produces responses in patients with moderate aplastic anemia. Blood 2003; 102: 3584-6
103. Blaise D, Olive D, Michallet M, et al. Impairment of leukaemia-free survival by addition of interleukin-2receptor antibody to standard graft-versus-host prophylaxis. Lancet 1995; 68: 1144-6
104. Hui CH, Sia H, Mangos H. Daclizumab has poor efficacy in steroid-refractory severe acute graft-versus-host disease: a single centre experience with 12 allograft patients [letter]. Bone Marrow Transplant 2008; 41: 409-10
105. Vincenti F, Kirkman R, Light S, et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. N Engl J Med 1998; 338: 161-5
106. Mottershead M, Neuberger J. Daclizumab. Expert Opin Biol Ther 2007; 7: 1583-96
107. Waldmann TA. Daclizumab (anti-Tac, Zenapax) in the treatment of leukemia/lymphoma. Oncogene 2007; 25: 3599-603
108. Willenbacher W, Basara N, Blau I, et al. Treatment of steroid refractory acute and chronic graft-versus-host disease with daclizumab. Br J Haematol 2001; 112: 820-3
109. San T, Moini H, Emerk K, et al. Protective effect of defibrotide on perfusion induced endothelial damage. Thromb Res 2000; 99: 335-41
110. Schroder H. Defibrotide protects endothelial cells, but not L929 tumour cells, from tumor necrosis factor-α-mediated cytotoxicity. J Pharm Pharmocol 1995; 47: 250-2
111. Sala M, Leone MP, Lampugnani P, et al. Polydeoxyribonucleotide (defibrotide) protects against post-ischemic behavioral, electroencephalographic and neuronal damage in gerbil. Eur J Pharmacol 1997; 328: 143-52
112. Sucak GT, Aki ZS, Yagci M, et al. Treatment of sinusoidal obstruction syndrome with defibrotide: a single-center experience. Transplant Proc 2007; 39: 1558-63
113. Chalandon Y, Roosnek E, Mermillod B, et al. Prevention of veno-occlusive disease with defibrotide after allogeneic stem cell transplantation. Biol Blood Marrow Transplant 2004; 10: 347-54
114. Falanga A, Vignoli A, Marchetti M, et al. Defibrotide reduces procoagulant activity and increases fibrinolytic properties of endothelial cells. Leukemia 2003; 17: 1636-42
115. Pogliani EM, Perseghin P, Parma M, et al. Defibrotide in recurrent thrombotic thrombocytopenic purpura. Clin Appl Thromb Hemost 2000; 6: 69-70
116. Rollins BJ. Hepatic veno-occlusive disease. Am J Med 1986; 81: 297-306
117. Bulley SR, Strahm B, Doyle J, et al. Defibrotide for the treatment of hepatic veno-occlusive disease in children. Pediatr Blood Cancer 2007; 48: 700-4
118. Besisik SK, Ozturk GB, Caliskan Y, et al. Complete resolution of transplantation-associated thrombotic microangiopathy and hepatic veno-occlusive disease by defibrotide and plasma exchange. Turk J Gastroenterol 2005; 16: 34-7
119. Bairey O, Kirgner I, Yakobi M, et al. Clinical severe hepatic venoocclusive disease during induction treatment of acute monoblastic leukemia managed with defibrotide. Am J Hematol 2002; 69: 281-4
120. Ho VT, Linden E, Revta C, et al. Hepatic veno-occlusive disease after hematopoietic stem cell transplantation: review and update on the use of defibrotide. Semin Thromb Hemost 2007; 33: 373-88
121. Sayer HG, Will U, Schilling K, et al. Hepatic veno-occlusive disease (VOD) with complete occlusion of liver venules after tandem autologous stem cell transplantation: successful treatment with high-dose methylprednisolone and defibrotide. J Can Res Clin Oncol 2002; 128: 148-52
122. Saviola A, Luppi M, Potenza L, et al. Late occurrence of hepatic veno-occlusive disease following gemtuzumab ozogamicin: successful treatment with defibrotide. Br J Haematol 2003; 123: 752-3
123. Corbacioglu S, Hönig M, Lahr G, et al. Stem cell transplantation in children with infantile osteopetrosis is associated with a high incidence of VOD, which could be prevented with defibrotide. Bone Marrow Transplant 2006; 38: 547-53
124. Dignan F, Gujral D, Ethell M, et al. Prophylactic defibrotide in allogeneic stem cell transplantation: minimal morbidity and zero mortality from veno-occlusive disease. Bone Marrow Transplant 2007; 40: 79-82
125. Corti P, Uderzo C, Tagliabue A, et al. Defibrotide as a promising treatment for thrombotic thrombocytopenic purpura in patients undergoing bone marrow transplantation [letter]. Bone Marrow Transplant 2002; 29: 542-3
126. Artesani MC. Anaphylactic shock to defibrotide [letter]. Allergy 2006; 61: 1022
127. Reddy PS, Deauna-Limayo D, Cook JD, et al. Rituximab in the treatment of relapsed thrombotic thrombocytopenic purpura. Ann Hematol 2005; 84: 232-5
128. Scully M, Cohel H, Cavenagh J, et al. Remission in acute refractory and relapsing thrombotic thrombocytopenic purpura following rituximab is associated with a reduction

in IgG antibodies to ADAMTS-13. Br J Haematol 2006; 136: 451-61

129. Ahmad A, Aggarwal A, Sharma D, et al. Rituximab for treatment of refractory/relapsing thrombotic thrombocytopenic purpura (TTP). Am J Hematol 2004; 77: 171-6
130. George JN, Woodson RD, Kiss JE, et al. Rituximab therapy for thrombotic thrombocytopenic purpura: a proposed study of the transfusion medicine/hemostasis clinical trials network with a systematic review of rituximab therapy for immune-mediated disorders. J Clin Apheresis 2006; 21: 49-56
131. Fakhouri F, Vernant JP, Veyradier A, et al. Efficiency of curative and prophylactic treatment with rituximab in ADAMTS13-deficient thrombotic thrombocytopenic purpura: a study of 11 cases. Blood 2005; 106: 1932-7
132. Au WY, Ma ES, Lee TL, et al. Successful treatment of thrombotic microangiopathy after haematopoietic stem cell transplantation with rituximab. Br J Haematol 2007; 137: 475-8
133. Ostronoff M, Ostronoff F, Calixto R, et al. Life-threatening hemolytic-uremic syndrome treated with rituximab in an allogeneic bone marrow transplant recipient [letter]. Bone Marrow Transplant 2007; 39: 649-51
134. Gallerani E, Lerch E, Romagnani E, et al. Thrombotic thrombocytopenic purpura associated with renal failure after autologous transplantation for multiple myeloma successfully treated with rituximab. Eur J Haematol 2006; 77: 527-9
135. Au WY, Lie A, Kwong YL. Rituximab in thrombotic microangiopathy: response to Castagna, et al. [letter]. Br J Haematol 2007; 139: 166-67
136. Carson KR, Evens AM, Rosen ST, et al. Progressive multifocal leukoencephalopathy associated with rituximab treatment of B-cell lymphoproliferative disorders: a report of 29 cases from the RADAR Project [abstract no. 370]. Blood 2007; 110 Suppl.: 116a
137. Schleintz N, Ebbo M, Mazodier K, et al. Rituximab as preventative therapy of a clinical relapse in TTP with ADAMTS13 inhibitor. Am J Hematol 2007; 82: 417-8
138. Kebriaei P, Saliba RM, Ma C, et al. Allogeneic hematopoietic stem cell transplantation after rituximab-containing myeloablative preparative regimen for acute lymphoblastic leukemia. Bone Marrow Transplant 2006; 38: 203-9
139. Takatsuka H, Takemoto Y, Iwata N, et al. Oral eicosapentaenoic acid for complications of bone marrow transplantation. Bone Marrow Transplant 2001; 28: 769-74
140. Yonezawa Y, Hada T, Uryu K, et al. Inhibitory effect of conjugated eicosapentaenoic acid on mammalian DNA polymerase and topoisomerase activities and human cancer cell proliferation. Biochem Pharmacol 2005; 70: 453-60
141. Chello M, Goffredo C, Patti G, et al. Effects of atorvastatin on arterial endothelial function in coronary bypass surgery. Eur J Cardiothorac Surg 2005; 28: 805-10
142. Patti G, Chello M, Pasceri V, et al. Protection from procedural myocardial injury by atorvastatin is associated with lower levels of adhesion molecules after percutaneous coronary intervention: results from the ARMYDA-CAMs (Atorvastatin for Reduction of Myocardial Damage during Angioplasty-Cell Adhesion Molecules) substudy. J Am Coll Cardiol 2006; 48: 1560-6
143. Boehme MW, Gao IK, Norden C, et al. Decrease in circulating endothelial cell adhesion molecule and thrombomodulin levels during oral iloprost treatment in rheumatoid arthritis patients: preliminary results. Rheumatol Int 2006; 26: 340-7
144. Bohm F, Settergren M, Gonon AT, et al. The endothelin-1 receptor antagonist bosentan protects against ischaemia/reperfusion-induced endothelial dysfunction in humans. Clin Sci (Lond) 2005; 108: 357-63
145. Mori T, Okamoto S, Watanabe R, et al. Successful treatment of bone marrow transplant-associated thrombotic microangiopathy with antithrombin-III. Am J Med 2002; 112: 81-2
146. Hochstetler LA, Flanigan MJ, Lager DJ. Transplant-associated thrombotic microangiopathy: the role of IgG administration as initial therapy. Am J Kidney Dis 1994; 23: 444-50

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