ENDOTHELIAL PATHOLOGY IN THROMBOTIC MICROANGIOPATHIES

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Introduction
Thrombotic microangiopathy is a term for a morphologic lesion characterized by platelet and fibrin thrombi involving the microvasculature. The morphologic lesions thus overlap between hemolytic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), and the lesions seen in scleroderma and malignant hypertension. HUS/TTP typically involves glomeruli and smaller vessels, whereas scleroderma and malignant hypertension involve interlobular arteries and arterioles. Hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) share the morphologic lesion of thrombotic microangiopathy, characterized by platelet thrombi occluding the microvasculature. The HUS and TTP syndromes overlap clinically. Recent evidence indicates differing pathogenesis (see below).

Clinical Features
The clinical features of essential hypertension complicated by malignant hypertension, scleroderma and classic HUS vary markedly. However, these entities may all have fibrin in the glomeruli and arterioles and interlobular arteries in the kidney. In essential hypertension, the level of blood pressure does not directly predict degree of end organ damage: African-Americans have higher risk for more severe end organ damage at any level of blood pressure. In malignant hypertension, fibrinoid necrosis primarily involves arterioles and interlobular arteries, contrasting the typical glomerular dominance of fibrin thrombi in classic HUS.

Scleroderma is a multisystem disease that affects the skin, the gastrointestinal tract, the lung, the heart and the kidney involvement occurs in approximately 60-70%. Scleroderma renal crisis, manifest by malignant hypertension, acute renal failure, some even with infarcts, develops in approximately 20% of patients with systemic sclerosis. Age at onset of systemic sclerosis is 30-50 years, and females are affected more than males.

HUS/TTP overlap clinically. TTP is more common in adults, and is characterized by fever, bleeding, hemolytic anemia, renal failure and neurologic impairment. Classic diarrhea-associated HUS is most common in infants and small children, and is characterized by acute renal failure, non-immune hemolytic anemia and thrombocytopenia. The renal manifestations at presentation include hematuria and low grade proteinuria with elevated creatinine in severe cases. Intravascular hemolysis is evident by increased bilirubin and LDH, reticulocytosis and low haptoglobin. Of note, thrombocytopenia may not be present by the time a renal biopsy is performed, especially in the transplant setting, where these manifestations of injuries in the kidney may be dysynchronous with peripheral blood manifestations.

Pathologic Features
Malignant hypertension/scleroderma
Microscopically, there is fibrinoid necrosis of afferent arterioles. Interlobular arteries show intimal thickening, proliferation of endothelial cells, and edema. RBC fragments are often present within the injured vessel wall, and there may be vessel wall necrosis and/or fibrin thrombi within vessels. Glomeruli may show ischemic collapse, or fibrinoid necrosis. In chronic injury, there is reduplication of the elastic internal lamina, so-called onion skin pattern. Tubules may show degeneration and even necrosis, especially in scleroderma crisis. Tubulointerstitial fibrosis develops with chronic injury. There are no immune complexes, and EM shows only increased lucency of the lamina rara interna, similar to chronic TMA (see below).

Thus, the pathologic appearance of scleroderma overlaps with malignant hypertension and thrombotic microangiopathy. Idiopathic malignant hypertension tends to involve smaller vessels, i.e. afferent arterioles, whereas scleroderma renal crisis may more often extend to interlobular size and larger
vessels, and TMA typically involves primarily glomeruli. However, distinction of scleroderma and malignant hypertension solely on morphological grounds is not often feasible, and clinicopathologic correlation is required for specific diagnosis.

**HUS/TTP**

Fibrin and platelet thrombi are present, primarily in the glomeruli. Fibrin is best visualized on hematoxylin and eosin or silver stains. Lesions may extend to arterioles, with some overlap with progressive malignant hypertension and scleroderma, where arteriolar and even larger vessel involvement occurs. At early stages, the glomeruli show thickened capillary walls due to fibrillar lucent material in the lamina rara interna of the basement membrane. This may result in a double contour basement membrane by light microscopy silver stains. Mesangiolysis occurs frequently, a subtle lesion, which may be overlooked. Mesangial areas seem to “unravel”, resulting in very long, sausage-shaped capillary loops due to the loss of mesangial integrity and coalescence of adjoining loops.

In infants and young children, thrombotic lesions predominate. In older children and adults, varied lesions occur. Many glomeruli may show only ischemic changes with corrugation of the glomerular basement membrane and retraction and collapse of the glomerular tuft. Segmental necrosis may be seen with rare well-developed fibrin thrombi. In severe cases, cortical necrosis with necrosis of tubules and glomeruli may occur. Secondary changes late in the course include glomerular sclerosis, either segmental or global. Reduplication of the glomerular basement membrane may occur in the late phase due to organization following endothelial injury. Arterioles and arteries, when involved, show thrombosis and sometimes necrosis of the vessel wall, with intimal swelling, mucoid change and later intimal proliferation. Fragmentation of red blood cells within the vessel wall may also be seen. Tubular and interstitial changes are proportional to the degree of glomerular changes.

Immunofluorescence studies show no immunoglobulin deposits. Complement and IgM may be present in sclerotic areas. Fibrin and fibrinogen are present in glomeruli and arterioles.

Electron microscopy reveals increased lucency of the lamina rara interna due to accumulation of fluffy material in the subendothelial space, a sign of endothelial injury. Endothelial cells are frequently swollen and detachment may be seen by electron microscopy. Mesangiolysis is a prominent finding in early phases. In the subacute phase, the increased lucency of the lamina rara interna is in part correlated to breakdown of coagulation products. This zone contains breakdown products of fibrin, laminin and fibronectin.

**Pathogenesis**

**Arterionephrosclerosis/malignant hypertension**

Arterionephrosclerosis lesions do not correlate directly with level of systolic blood pressure. It is possible that underlying microvascular disease causes the hypertension and the renal disease in susceptible patients. Underlying causes include possible genetic and structural components, such as decreased nephron number, perhaps linked to low birth weight, even in normal African Americans versus Caucasians. Most recently, polymorphisms in nonmuscle myosin heavy chain (MYH9) have been linked to excess risk of sclerosis in hypertension and HIVAN in African Americans. This protein is expressed in podocytes, and platelets, and appears to be important for actin cytoskeleton in podocyte foot processes. Malignant hypertension is postulated to develop if usual hypertension remains untreated with direct hemodynamic injury causing endothelial injury and fibrinoid necrosis.

**Scleroderma**

The pathogenesis of scleroderma is probably immune with unknown inciting events. Autoantibodies are often present, including anti-topoisomerase I, anticentromere, anti-RNA polymerase, each present in 25%, and only one of these positive in any one patient. Imbalance of vasodilators (e.g. nitric oxide, vasodilatory neuropeptides such as calcitonin gene-related peptide, substance P) and vasoconstrictors (e.g. endothelin-1, serotonin, TXA2) has been described in scleroderma patients. Endothelial injury and impaired vasculogenesis are thought to play key roles in renal scleroderma. Early lesions include endothelial cell apoptosis with large gaps between endothelial cells, loss of integrity of the endothelial lining, and vacuolization of endothelial-cell cytoplasm, with proliferation of pericytes and vascular smooth muscle cells, with later increase in fibroblasts in the interstitium. In addition, arterioles show several basal lamina-like layers, perivascular infiltrates of mononuclear immune cells, obliterator microvascular lesions, and later rarefaction of capillaries. Fibrosis is likely contributed to by increased endothelin-1, TGF-beta, and PDGF, among other growth factors.
HUS/TTP
Numerous etiologies for HUS/TTP exist. We will review pathogenesis of classic, D+ and atypical HUS, infection and drug-related mechanism of injury.

Classic, D+ HUS. Recent increased understanding has allowed some separation of most common etiologies underlying HUS and TTP, although much overlap remains. Typical diarrhea associated (D+) HUS is caused by shiga toxin from pathogenic strains of Escherichia coli 0157:H7. Verotoxin was associated with ~90% of cases of HUS in children in North America and Europe. Undercooked hamburger meat is most closely associated with such outbreaks in North America, pointing to cattle as an important reservoir for the implicated E. coli serotype 0157:H7. In addition, this E. coli strain can be transmitted from person-to-person, and outbreaks associated with swallowing contaminated lake water, or ingestion of contaminated fruit or vegetables or cider have occurred.

The mature verotoxin has alpha and beta subunits. The A subunit has ricin-like N-glycosidase activity and the B subunits form a multimer responsible for binding to membrane glycolipids, globotriaosyl ceramide (Gb3), globotetraosyl ceramide and a blood group glycolipid AgP3, that comprise the shiga toxin receptors. The translocated intimin receptor (Tir) is inserted into the plasma membrane of the host enterocyte, and E. coli is anchored to the cells, followed by bloody diarrhea in nearly all patients. The alpha unit is cleaved and taken up by endocytosis, inactivating 60S ribosomes, thereby causing cell death. The Gb3 receptor for verotoxin is highly expressed in human kidney, perhaps underlying the susceptibility of the kidney to this toxin. However, Gb3 levels were not different in normal children vs adults, so the excess risk of children for D+ HUS cannot be simply explained by overexpression of Gb3.

In response to shiga toxin, fractalkine (FKN), a CX3C transmembrane chemokine, acts as an adhesion counterreceptor on endothelial cells and a chemoattractant. This induction plays an essential role in promoting leukocyte-endothelial cell interaction and may contribute to renal microvascular dysfunction. Whether this pathway is particularly upregulated in children remains to be determined. The microvascular endothelium is log orders more sensitive to the toxin than large vessel endothelium. The kidney endothelium is particularly sensitive to the toxin. Toxin exposure causes a proadhesive and prothrombotic change in the microendothelium, even at doses below those resulting in cell death, mediated through effects on protein synthesis. Particularly interesting is the stabilization by toxin of endothelin-1 mRNA, a transcript that is normally labile. A verotoxin-response element in the 3'-UTR of the ET-1 gene allows toxin to stabilize ET-1 mRNA interaction with polyribosomes.

Atypical HUS/complement/vWF factor dysregulation. Atypical, diarrhea negative (D-) HUS may often be due to abnormalities in complement dysregulation or abnormalities in von Willebrand factor function. Complement activation is normally controlled by complex interplay of various membrane-bound and soluble factors; such as factor I, which normally dissociates activated C3b. When these regulatory factors are dysfunctional, vast excess of C3b is deposited by amplification. Inheritance has variable penetrance and may be autosomal recessive or dominant. Frequency of mutations of complement regulatory proteins in atypical HUS w-varies, with complement factor H most common (CFH, 20-30%). Other mutations include CFHR1/3 (6%), complement factor I (CFI, 4-10%) or B (CFB (1-2%) , C3 (5-10%), thrombomodulin (THBD, 5%) or membrane cofactor protein, MCP (10-15%). Prognosis varies according to the mutation involved, with best prognosis with MCP mutations, and worse with complement factor H mutations, with mutations of CFI and C3 intermediate.

TTP patients often have abnormalities of the vWF-cleaving protease ADAMTS13 (a member of the "a disintegrin and metalloprotease with thrombospondin type 1 repeats" family of zinc metalloproteases). Absence of ADAMTS 13 results in large von Willebrand factor multimers, which promote platelet aggregation and thrombosis. Patients may have inherited deficiency of ADAMTS13, usually inherited in an autosomal recessive manner, or secondary acquired inhibitors with antibodies to ADAMTS13, which may be triggered, by drugs or infection.

HIV Infection. Thrombotic microangiopathy may also occur in other infections including HIV. This complication is a common cause of acute renal failure in HIV positive patients. The pathogenesis is poorly understood. Direct infection of renal endothelial cells with HIV has not been shown, and the renal microvasculature lacks expression of CD4 and other co-receptors such as CCR5 and CXCR4 that mediate HIV infection in leukocytes. Some data suggest that HIV variants and peptide subunits can cause apoptosis in microvascular endothelial cells in vitro. The HIV envelope protein gd120 can induce expression of the procoagulant tissue factor in human arterial smooth muscle cells. However, these mechanisms have not been shown to be operative in vivo. Macaque monkeys infected with HIV -2 developed thrombotic microangiopathy with widespread apoptosis of endothelial cells with TUNEL positivity, an injury pattern referred to as tunelosis.
Drugs. Numerous drugs have also been associated with development of thrombotic microangiopathy including calcineurin inhibitors, antagonists of vascular endothelial growth factor (VEGF), anti-platelet drugs and chemotherapeutic drugs.

Glomerular cell interaction and TMA: anti-VEGF antibodies. Injury of the mesangium with dissolution of matrix and loss of cells is a characteristic finding seen early on in many thrombotic microangiopathies and may result in microaneurysms. This injury pattern is frequently also seen in diabetic nephropathy, illustrating the interplay of glomerular cells in producing the final phenotype of injury. Interestingly, in diabetic nephropathy, local increased red blood cell fragmentation was associated with increased PAI-1, further pointing to local microvascular injury.

The podocyte is crucial for maintaining endothelial cell survival. Elegant studies by the group of Quaggin have pointed to the necessity of having the correct balance of VEGF, produced by podocytes, to optimize endothelial cell survival. This principle is illustrated by the occurrence of thrombotic microangiopathy in a small subset of patients receiving cancer therapy with antagonism of VEGF, such as bevacizumab. Local deficiency of VEGF induced in adult mice by podocyte knockout of VEGF resulted in thrombotic microangiopathy. These results illustrate the importance of the podocyte for maintenance of normal glomerular integrity. The fenestrated glomerular endothelium is dependent upon induction of VEGF expression. Loss of VEGF does not allow formation of the normal fenestrated phenotype, a key component of the normally functioning microvasculature in the glomerulus. Importance of the VEGF pathway for endothelial injury is also seen in preeclampsia, where increased soluble receptor for VEGF, sFlt (soluble fms-like tyrosine kinase) is observed, likely arising from ischemic injury arising in the placenta. The increased levels of sFlt bind and inactivate VEGF and placental growth factor, with resulting endothelial injury in the glomerulus. Rodents with excess sFlt develop features of preeclampsia, including the classic endotheliosis lesion, with swollen endothelial cells and a bloodless appearance of the glomerulus.

Other drugs- chemotherapy, calcineurin inhibitors, anti-platelet drugs. Other chemotherapeutic drugs also associated with thrombotic microangiopathy, including mitomycin and gemcitabine. A peculiar feature of mitomycin is the long delay after exposure until appearance of thrombotic microangiopathy, perhaps resulting from the dose dependency of injury and the slow turnover of glomerular endothelial cells. These drugs are thought to involve direct toxicity to endothelial cells. Similar mechanisms are thought to play a role with calcineurin inhibitors, which may also injure the media if given in high doses, resulting in the nodular insudation extending to the media and hyalinosis characteristic of calcineurin inhibitor toxicity. Anti-platelet agents such as clopidogrel and ticlopidine also directly activate and are toxic to endothelial cells. Acute immune-mediated injury may also occur with some anti-platelet agents with antibodies directed to platelets, such as can occur with quinine. Rarely, anti-platelet agents may also result in antibodies produced against ADAMTS13. Thus in sum, the mechanisms of drug induced injury are diverse, including endothelial toxicity, immune mechanisms with some patients developing auto-antibodies, and perturbation of the podocyte-endothelium interaction that is key for glomerular homeostasis.

Lupus anticoagulant/anti-phospholipid antibody. TMA may occur in patients with SLE, often but not always associated with anti-cardiolipin or anti-phospholipid antibodies. Antiphospholipid syndrome also occurs in patients without SLE. The morphology of the TMA is not unique, but coexistence of TMA with immune complex disease with features of lupus nephritis should suggest possible APL as an etiology. Isolated APL syndrome in the kidney results in a vascular nephropathy characterized by small vessel vaso-occlusive lesions associated with fibrous intimal hyperplasia of interlobular arteries, recanalizing thrombi in arteries and arterioles with resulting focal sharply demarcated cortical atrophy. Glomerular and arteriolar TMA lesions may also occur.

Rejection. Acute humoral rejection may also have endothelial injury and fibrinoid necrosis of arteries, most often with positive C4d as a marker of antibody binding to the peritubular capillaries, the primary target of this injury. In a study of platelet aggregation in various transplant biopsies, intracapillary platelet activation was found related to injury from many causes and was not specific for antibody-mediated rejection.

Animal models. Further insight and understanding of mechanisms in HUS/TTP have been hampered by a lack of suitable animal models. Radiation injury in the rodent may produce some features mimicking thrombotic microangiopathy, with early endothelial injury, fibrin thrombi followed by organization of lesions and increased scarring. This progressive renal injury was sensitive to inhibition of angiotensin with associated marked decrease in plasminogen activator inhibitor-1 (PAI-1). However, in a mouse model of HUS with intraperitoneal injection of shiga toxin- II and lipopolysaccharide, PAI-1 deficiency did not modify
morbidity or mortality, but was associated with increased inflammation and increased expression of TNF-alpha. Of note, this model is limited in that there mainly is tubular injury with minimal fibrin thrombi, although peripheral blood hemolysis and thrombocytopenia was observed, and acute renal failure developed.

In vitro studies of human glomerular endothelial cells support that local renin angiotensin system activation may be important in promoting injury. Cells exposed to angiotensin showed increased tissue factor activity when they also were injured with TNF-alpha and shiga toxin 1, and with less injury when treated with either angiotensin receptor blocker or angiotensin converting enzyme inhibitor. Recently, a novel mouse model of HUS was reported. Injury was induced by selective renal arterial perfusion with the lectin concanavalin A (Con A) followed by specific anti-Con A antibody. Glomerular and peritubular capillary microvascular thrombosis developed with renal failure and presence of schistocytes. Although the initial pathogenic insult differs from that typically seen in humans, this model could prove useful in establishing secondary mechanisms of injury.

Clinicopathologic Correlations and Prognosis of HUS/TTP lesions
Histological distribution of lesions may have some prognostic significance (see below). Age has a major impact on prognosis. Mortality of TTP in adults was nearly 100% before advent of plasma therapy. Children have a much more benign course, with less than 10% mortality even when only symptomatic treatment was given. Improved survival in the last ten years is associated with use of a combination of anti-platelet agents and plasmapheresis. In some series, plasma exchange has resulted in better prognosis than plasma infusion, but the results are not clear-cut. HUS accounts for about half of cases of acute renal failure in HIV patients, and has a poor outcome.

Long-term follow-up 10 years after HUS has shown decrease in GFR in half of patients. Histological distribution of lesions may have some prognostic significance. Degree of histologic damage rather than initial clinical severity was the best predictor of long-term prognosis in HUS. Predominantly glomerular involvement has a better outcome than larger vessel involvement. Glomerular predominant injury is the most frequent pattern of injury in children. Hypertension is more frequent with larger vessel, rather than glomerular, injury. Poor prognosis was predicted by cortical necrosis or thrombotic microangiopathy involving >50% of glomeruli at time of presentation. Segmental sclerosis was associated with decreased GFR long term. Recurrence in the transplant is very common in familial forms of HUS, and is most often associated with graft loss, except for patients with MCP mutation, as this factor is produced in the kidney, and the normal transplant may thus provide sufficient factor to normalize function. Other complement regulatory factor mutations may require liver transplant for cure. Initial levels of serum plasminogen activator inhibitor-1 (PAI-1) in patients with HUS also correlated with worse long-term outcome, perhaps because high PAI-1 promotes thrombosis and also inhibits matrix breakdown.

REFERENCES


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