Microangiopathic hemolytic anemia (MAHA)

BCSLS Congress 2012
Kamloops, BC

Dr. Gerry James
Royal Inland Hospital
Kamloops, BC
Interior Health
Disclosures

• no relevant disclosures
MAHA

• Definition
• Classic types of disorders with MAHA
  – Pathophysiology
  – Clinical Presentation
  – Laboratory Findings
  – Basic overview of their treatment
• Case presentation
• Summary
Definition

• MAHA is a type of hemolytic anemia that occurs in numerous, but not all, conditions known as the “thrombotic microangiopathies (TMA)”

• Though diverse, TMAs are somewhat similar in that many involve endothelial injury and clotting activation in the microvasculature.

• the pathophysiology of the underlying disorder many times leads to microthrombi in capillaries and arterioles that results in end organ injury usually by ischemia
Definition

• the hemolysis that occurs in MAHA is due to physical destruction of the red blood cell in the small blood vessels as they pass by the microthrombi

• characterized by red cell fragmentation and associated with peripheral thrombocytopenia
Types of TMAs associated with MAHA

• Thrombotic thrombocytopenic purpura (TTP)
• Hemolytic uremic syndrome (HUS)
• Other TMA syndromes can occur with:
  – Disseminated intravascular coagulation (DIC)
  – Pregnancy
  – Cancer and chemotherapy
  – HIV infection
  – post hematopoietic stem cell transplant
  – drugs (ie. quinine)
Thrombotic thrombocytopenic purpura (TTP)

- first described by Moschcowitz in 1925 as an acute febrile pleiochromic anemia with hyaline thrombi in the brain, pancreas, spleen, kidney, and adrenal gland.
- rare
- untreated – high mortality rate approaching 90%
TTP Pathophysiology

- ultimate cause is unknown
- endothelium damage (possibly from infection) and activation of clot formation may be involved as an initiation event
- vonWillebrand factor (vWF) plays a central role in the pathogenesis of TTP
  - a large multimeric protein involved in the initiation of platelet clumping
- patients with TTP lack a protease enzyme that is essential in the breakdown of ultralarge vWF multimers
- ADAMTS13 – a disintegrin-like and metalloprotease with thrombospondin type 1 motif 13
Platelets play an essential role in orchestrating the blood clotting processes necessary to seal breaches in the endothelium of blood vessels that may be caused through injury. Platelets circulate in an inactive form for a 9-10 days before removal by the spleen. Recruitment and activation of platelets at sites of endothelium damage is dependent on cellular factors, such as von Willebrand factor, produced by endothelial cells.
Endothelial cells near the site of damage respond by synthesising von Willebrand factor which is secreted in the form of large multimeric chains. Platelets express cell surface receptors, such as GP1b, that allow them to adhere to von Willebrand factor bound to subendothelial collagen fibrils.
ADAMTS13 cleaves vWF
Platelets are recruited to sites of endothelium damage by detection of von Willebrand factor bound to exposed subendothelial collagen, using cell surface GPIb receptors. They can also directly bind to subendothelial collagen fibrils, using cell surface GPIV receptors and \( \alpha 2\beta 1 \) integrins. In addition, platelets bind other platelets, through GPIIb/IIIa receptors that recognise fibrinogen as an intermediate. In this way platelets aggregate to form a seal the breach in the endothelium and initiate the blood clotting cascade that generates a meshwork of insoluble fibrin.
In the absence of ADAMTS13 proteolytic activity, there are higher levels of the large multimeric chains of von Willebrand factor in circulation that are able to bind to exposed subendothelial collagen fibrils and initiate recruitment of large numbers of platelets to sites of endothelium damage.
Thrombotic Thrombocytopenic Purpura

Platelets express cell surface GPIb receptors that recognise von Willebrand factor bound to collagen fibrils exposed at the site of endothelium damage. In TTP, the large multimeric chains of von Willebrand factor recruit and activate excessive numbers of platelets, which in turn leads to platelet depletion (thrombocytopenia). The large aggregation of platelets also impedes the passage of erythrocytes through small blood vessels and can cause the cells to shear, resulting in anaemia and organ ischaemia. Fragments of erythrocytes are visible in blood smears and are known as schistocytes.
TTP clinical presentation

• classic “pentad” (only found in 40% of cases)
  – MAHA
    • hemolytic anemia – fatigue
    • thrombocytopenia - petechiae
  – fever
  – renal insufficiency
  – neurological symptoms – mental status, seizures, paresthesias, visual disturbances, aphasia (84-92% of cases)

• other findings
  – thrombosis and hemorrhage
  – cardiac signs (elevated troponin, conduction abnormalities, etc.)
  – pancreatitis, lung, ocular injury
Histopathology of TTP (brain)

Microthrombi are composed primarily of platelets and vWF
Histopathology of TTP (cardiac muscle)

Microthrombi are composed primarily of platelets and vWF (very little fibrin present)
TTP Laboratory findings

• Complete blood count shows:
  – anemia
  – thrombocytopenia (often critically low – 20,000 or less)
  – elevated reticulocyte count

• Chemistry
  – elevated bilirubin (100% of cases)
  – elevated LDH (more than a 1000)
  – low haptoglobin level
  – decreased renal function tests (30% of cases – usually mild)
  – mild proteinuria and hematuria
Peripheral smear in microangiopathic hemolytic anemia showing presence of schistocytes

Peripheral blood smear from a patient with a microangiopathic hemolytic anemia with marked red cell fragmentation. The smear shows multiple helmet cells (small black arrows), other fragmented red cells (large black arrow); microspherocytes are also seen (blue arrows). The platelet number is reduced; the large platelet in the center (red arrow) suggests that the thrombocytopenia is due to enhanced destruction.

Courtesy of Carola von Kapff, SH (ASCP).
TTP laboratory findings

• Coagulation tests:
  – Prothrombin time/INR – usually normal
  – activated partial thromboplastin time – usually normal
  – vWF multimers – excessive levels of large molecular weight multimers by Western blot analysis
TTP forms

- acquired autoimmune (most common)
  - adults (F>M); usually in the fourth decade
  - ADAMTS13 activity <10%
  - due to an autoantibody that binds ADAMTS13
  - association with autoimmune disease

- congenital (rare)
  - usually seen in childhood though may present at any age
  - chronic, relapsing
  - due to a congenital deficiency of ADAMTS13
TTP Treatment
TTP can be treated by plasma infusion or exchange therapy which provides the missing enzyme ADAMTS13 and restores proteolytic cleavage of the large multimeric chains of von Willebrand factor into smaller fragments. Plasma exchange therapy additionally contributes to the removal of the ADAMTS13 inhibitor, such as autoantibodies.
Corticosteroids, cyclosporine are also used.

In some cases plasma infusion or exchange therapy fails to provide a long-term benefit. When autoantibodies are responsible for depletion of ADAMTS13, this is likely a result of the persistence of autoantibody producing B lymphocytes that continue to secrete autoantibodies. Rituximab is an CD20-binding monoclonal antibody that selectively binds to B lymphocytes and mediates their destruction. In HIV-infected people, Rituximab has been used successfully without evidence of clinical worsening or increased opportunistic infection.
Importance of recognizing MAHA early

- TTP will be considered in the differential diagnosis

- survival now approaching 90% with PEX, corticosteroids, beginning use of rituximab since 2008 (Am. J. Hematol. 87:S88-S91, 2012)

- N.B. Avoid platelet transfusion as it can aggravate the disease course
Hemolytic uremic syndrome (HUS)
HUS

- is a TMA that is characterized by the triad of hemolytic anemia, peripheral thrombocytopenia (MAHA) and renal failure in 100% of cases

- Forms:
  - typical (Diarrhea positive – D+HUS)
  - atypical (5-10%) D-HUS
Typical HUS

• Diarrhea positive (D+ HUS)

• usually occurs after intestinal infection with Shiga-toxin-producing bacteria
  – *E. coli* O157:H7 (shigatoxins 1 and 2)
  – *Shigella dysenteriae* serotype 1

• most patients are <3 years of age though can occur in adults
HUS Clinical findings

- Shiga-toxin producing bacterial strains can contaminate unpasteurized milk/dairy products, ground beef (ie. hamburger disease), agricultural produce, insufficiently cooked foods as well as municipal or swimming water.
- Diarrhea occurs 2-12 days later becomes bloody 1-2 days after that
- Intense abdominal pain and painful defecation.
D+ HUS Pathophysiology

- the bloody diarrhea occurs as a result of toxin induced colonic epithelial cell injury

- eventually the toxin enters the bloodstream and transported to the kidneys by neutrophils and monocytes
Fig. (4). Pathophysiological mechanisms leading to microthrombi formation in diarrhea-associated HUS. Shigatoxins are transported in blood flow by neutrophils, platelets and monocytes, and bind their receptors (globotriaosyl ceramide) at the surface of renal endothelial cells. IL-1, IL-6 and TNF-α up-regulate expression of shigatoxins receptors on endothelial cells surface. After internalization, they interfere with protein traduction machinery and thereby induce endothelial cell apoptosis. Damaged cells express surface high molecular weight VWF, which initiates platelet clumping through interaction with glycoprotein Ib. Shigatoxins also induce tissue factor expression on endothelial cells, leading to factor VII activation and fibrin formation. VWF: von Willebrand factor.
Renal lesion in fatal hemolytic-uremic syndrome

Thrombus (arrow) in a glomerular arteriole in a child who died with fulminant postdiarrhoeal hemolytic-uremic syndrome.

HUS - other clinical findings besides renal failure

- cerebral manifestations (cerebral edema, leukoencephalopathy, seizure, coma) – 50% of cases
- cardiac dysfunction
- intestinal complications (necrosis, perforation)
- pancreatitis
Treatment

• D+ HUS
  – symptomatic treatment – hemodialysis, blood pressure control medications, fluid management
  – antibiotics, antimotility agents, narcotics should be avoided as they are associated with worsening of the disease
  – NSAIDS should be avoided as they can decrease renal blood flow
Prognosis

– usually good prognosis

– acute renal failure and death – 12% of cases

– 25% that have acute renal failure have long term renal sequelae
Atypical HUS

- Diarrhea negative (D- HUS)
- 5-10% childhood HUS though can rarely occur in adults
- associated with congenital dysfunction in the complement pathway involving mutations of several complement factors/proteins involved in regulation of the alternative pathway.
- rarely acquired due to autoantibodies directed against these same complement regulatory proteins
D- HUS Pathophysiology

- Complement proteins are part of our immune system that act in a controlled way to destroy invading microorganisms.

- Normally, these complement proteins are regulated by other proteins (ie. Factor H, I, CD46/MCP) so our own cells are not destroyed.

- If there are genetic mutations (or rarely, loss of action by an autoantibody) in these regulator proteins our own cells come under attack by excessive activation of the complement pathway.
D- HUS Pathophysiology

• in D-HUS, there is renal endothelial cell damage and the formation of fibrin microthrombi.

• Microthrombi lead to MAHA and renal ischemia/failure.
Treatment

• D- HUS

  – PEX benefits about a third of children except one subgroup (MCP mutations)

  – targeted therapy replacing deficient regulator proteins (ie. Factor H/I) may possibly occur in the future
Prognosis

– acute renal failure and death – 54% of cases

– \( \frac{1}{2} \) survivors have many relapses and 1/3 require long term dialysis

– depending on the deficient factor – the relapse rate varies and relapse can occur even after renal transplantation
Other TMA syndromes that may be associated with MAHA

- Pregnancy and post partum
  - HELLP syndrome
  - pre-eclampsia, eclampsia
- Hematopoietic stem cell transplantation
- Medications
- Disseminated intravascular coagulation
- Cancers
Pregnancy and post partum

- TMA in this cohort may show features of either TTP as well as HUS or HELLP syndrome

- HELLP stands for hemolysis, elevated liver enzymes, and low platelets

- Evidence of liver cell injury, DIC, and detectable ADAMTS13 may suggest HEELP rather than TTP

- HEELP/severe eclampsia – possibly due to maternal endothelium dysfunction

- Treatment depends on diagnosis:
  - HEELP – fetal extraction/delivery
  - TTP – PEX
Hematopoetic stem cell transplantation

- TMA may triggered by numerous factors
  - total body irradiation in transplant conditioning, infections, medications (ie. calcineurin inhibitors), graft versus host disease
- poor response to plasma exchange
- treatment targets the underlying factors
- defibrotide (ssPolynucleotide) may protect endothelial cells from cytokine mediated cell death
Medications

- large number of drugs implicated in TMA
  - antiplatelet agents (ie. ticlopidine, clopidogrel)
    - induces anti-ADAMTS-13 antibodies
    - 1 in 1600 to 5000 patients treated
  - antineoplastic drugs (ie. mitomycin C)
    - endothelial injury
  - quinine
    - induces quinine-dependent, platelet reactive antibodies
    - older women, abrupt onset
Disseminated intravascular coagulopathy

- pathological imbalance in the procoagulant and anticoagulant systems that lead to unregulated thrombin generation

- secondary to many states including sepsis/infection, trauma, toxins, malignancy, complications of pregnancy, burns, etc.

- eventually leads to the formation of fibrin microthrombi
Disseminated intravascular coagulopathy

• hemolytic anemia is seldom present but a MAHA picture may be present

• common question by the treating clinician is if the diagnosis is DIC or TTP
  – coagulation tests may be helpful
    • hypofibrinogenemia, prolonged aPTT and INR
  – can be difficult at times because of overlapping clinical associations
Case

- 58F
- diagnosed with invasive ductal adenocarcinoma in right breast in 2005
- hx of hypertension and osteoarthritis
- on antihypertensive medication
- admitted on March 28, 2011 with dizziness and pancytopenia
- c/o extreme fatigue and dyspnea
- developed dramatic onset of jaundice and assessed by GI service
• Physical exam:
  – lower leg edema, jaundice, tender and enlarged liver

• Imaging:
  – Bone scan showed diffuse osteoblastic disease
• Lab:
  – CBC
    • **Hemoglobin 62 g/L**
    • WBC $7.4 \times 10^9$/L
    • **platelet count 39 x10^9/L**
  – Coagulation
    • essentially normal INR, PTT, and fibrinogen
  – HIV – non reactive
  – Chemistry
    • increased total **bilirubin** (mainly conjugated), lipase, alk phos, and γGT
    • **Haptoglobin <0.20 g/L**
    • LDH 2993 U/L Day 2 rose to 4031 U/L Day 4
    • CA15-3 : 2907
Peripheral smear

- Microangiopathic picture
- Polychromasia, schistocytosis, occasional spherocytes and thrombocytopenia
Peripheral smear – showing normoblastemia

Leukoerythroblastic picture as well.
• Direct antiglobulin test – negative (Days 4 and 5)
• pRBC transfusion
  – Day 1 - 3 units
  – Day 2 – 2 units
  – Day 4– 1 unit (minor allergic trxn)
  – Day 5 – 1 unit (minor allergic trxn)

• Started on high dose corticosteroids

• Bone marrow examination was requested by oncology to assess for metastatic breast cancer
  – day 4
    • extremely difficult due to hardness of bone
    • dry tap with osteosclerosis and necrotic tissue
Core biopsy from second attempt (Day 5) under CT guidance (dry tap)

Conclusion: Osteosclerosis with fibrosis and crushed cells
Bone marrow core biopsy

No hematopoetic elements or fat.
Myelofibrosis and crushed cells with a single file arrangement
Second attempt under CT guidance (day 5) – dry tap

Crushed cells are positive for Pancytokeratin via IHC c/w metastatic carcinoma

Reticulin stain
Hospital course

• oncology service - very poor prognosis
• patient died suddenly on Day 7
• no autopsy performed
• combination of a MAHA picture with normoblastemia, and metastatic carcinoma is compatible with cancer associated thrombotic microangiopathy.
Cancer associated TMA

- stomach, breast and prostate cancers
- insidious onset
- dyspnea, weight loss, severe DIC with dacrocytes, massive erythromyелеmia are specific features.
- Bone marrow exam shows metastases
- Pathophysiology can be due to either:
  - tumor microemboli leading to microvascular occlusion
  - cytokines (ie. TNFα) leading to endothelial injury
  - acquired ADAMTS-13 deficiency (paraneoplastic syndrome)
- poor prognosis
Summary

• MAHA is not really a unique clinical entity but has many causes with different pathological mechanisms.
• MAHA is seen in many, though not all, thrombotic microangiopathic processes.
• The presence of anemia with schistocytes and thrombocytopenia are key features in recognizing this type of hemolytic process.
Summary

• MAHA is an extremely important pattern for the laboratorian to recognize as the underlying condition, though variable, can be rapidly fatal in many cases if left untreated.

• Finally, once recognized and promptly communicated to the treating physician, the patient’s treatment can be tailored for the best outcome.
Thank you!