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Letter to the Editor

A Thought on Possible Pathogenesis of Ebola Viral Hemorrhagic Disease and Potential Treatments: Could it Be Thrombotic Thrombocytopenic Purpura-Like Syndrome?

Dear Editor,

Ebola viral disease (EVD) is characterized by a febrile illness, with profound gastrointestinal manifestations, including nausea, vomiting, diarrhea, abdominal pain and gastrointestinal bleeding, and is complicated by intravascular volume depletion, shock and profound electrolyte abnormalities and organ dysfunction. The hemorrhage of viral hemorrhagic fever is a late manifestation and occurs only in a minority of patients according to field physicians from West Africa (1).

There is no known effective antiviral treatment for EVD, and many patients have concomitant malarial infection, which also has to be treated and may influence outcomes (1). When some patients progress to Ebola viral hemorrhagic disease (EVHD) despite standard supportive care, hydration, electrolyte replacement and good nutrition, increasing thrombocytopenia and changing coagulation profile could occur with progressive vital organ dysfunction. Therefore, for each patient to attain a favorable outcome, identifying the hematologic diagnosis and understanding its pathogenesis are essential to provide proper hematologic management prior to the onset of irreversible multi-organ failure (MOF).

Proposed pathogenesis

Like other known hemorrhagic viral diseases, Ebola viral infection can cause the endothelial cells (EC) toxicity through the mediation of the viral envelope glycoprotein, which activates EC and induces their functional changes (2–4).

To explain both acute inflammation and hematologic complication, a pathogenesis based on the activation of two pathways following an injury of EC is proposed as shown in Figure 1. One is inflammatory pathway and the other is microthrombotic pathway. The alteration of EC activates the inflammatory pathway through increased expression of various cytokines causing cytokine storm and also activates the microthrombotic pathway through suppression of cellular adhesion molecules compromising EC barrier function. The cytokine storm causes acute inflammation, and diminished barrier function prompts an excessive release of von Willebrand factor multimers (VWF). The cytokine storm leads to inflammatory manifestations of fever, chills, sweating, myalgia, arthralgia, weakness, dehydration and prostration.

The proposed pathogenesis of EVHD is based on hematologic changes that could occur due to activation of microthrombotic pathway. The likely culprit, causing pathologic changes in microcirculatory system (5), is the flooding of unusually large VWF (ULVWF), emerging from compromised EC barrier function. ULVWF plays an important role in vascular microthrombogenesis by activating and aggregating with platelets. Vascular microthrombosis would occur if protease ADAMTS13, which cleaves VWF multimers, is underexpressed or lacks sufficient activity in some people due to yet unidentified mechanism(s). One suggested mechanism might be mild to moderate phenotype expression of single nucleotide polymorphism of ADAMTS13 gene as seen in patients with cerebral malaria (6). The biological features of EC activation, abnormal circulating ULVWF, and significant reduction of ADAMTS13 in falciparum malaria (7) are similar to those caused by thrombotic thrombocytopenic purpura (TTP) (8).

An incomplete cleavage of ULVWF by insufficient ADAMTS13 would provoke aggregation of activated platelets, in which ULVWF becomes a bridge to form a complex of platelets-ULVWF and adheres to EC to cause vascular microthrombosis, resulting in thrombocytopenia and thrombotic microangiopathy (8). EVD-induced vascular microthrombosis could contribute to ischemic injury of vital organs, including the brain, lungs, liver and intestines. In TTP-like syndrome, ischemic injury due to vascular microthrombosis could cause central nervous system dysfunction (CNSD), acute respiratory distress syndrome (ARDS) (9), acute hepatitis and acute hepatic necrosis syndrome (AH/AHNS) (10–12), and gastro-intestinal hemorrhagic syndrome (11). Indeed, clinical features similar to these syndromes have been described in EVD, which presentations also fit with

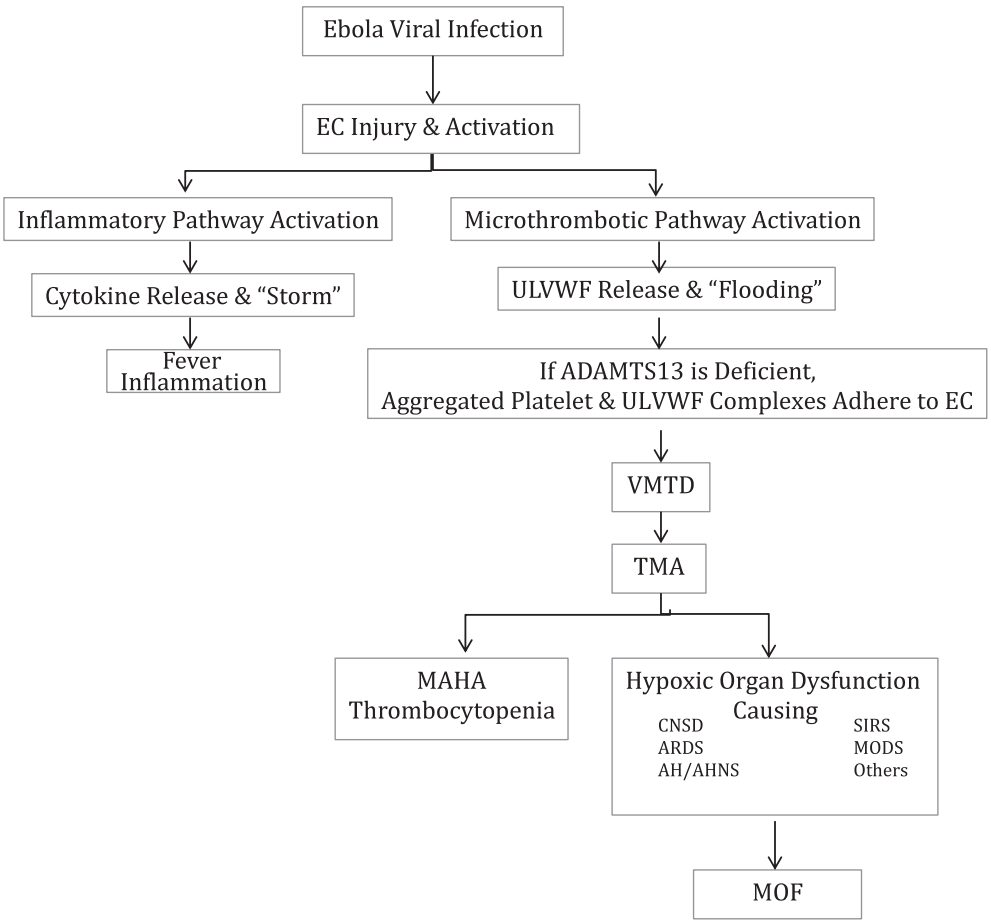

FIG. 1. Proposed pathogenesis of Ebola viral disease (EVD). AH/AHNS, Acute hepatitis/Acute hepatic necrosis syndrome; ARDS, Acute respiratory distress syndrome; CNSD, Central nervous system dysfunction; EC, Endothelial cell; MAHA, Microangiopathic hemolytic anemia; MODS, Multi-organ dysfunction syndrome; MOF, Multi-organ failure; SIRS, Systemic inflammatory response syndrome; TMA, Thrombotic microangiopathy; ULVWF, Unusually large von Willebrand factor multimers; VMTD, Vascular microthrombotic disease.

systemic inflammatory response syndrome (SIRS). SIRS is an inflammatory state affecting the whole body, frequently a response of the immune system to an infection or noninfectious insult. It is commonly seen in sepsis, and is known to show endothelial damage and vascular microthrombosis in animal models (13). These observations suggest that the manifestation of EVHD and SIRS could have been due to underlying TTP-like syndrome. All of above clinical syndromes eventually may lead to multi-organ dysfunction syndrome (MODS) and MOF.

Hematologic features may have two components: (i) consumptive thrombocytopenia due to platelet aggregation; and (ii) microangiopathic hemolytic anemia (MAHA) due to shear stress in arteriolar capillaries. In animal models, hematological and biochemical monitoring of EVD in rhesus monkeys have shown MAHA, thrombocytopenia and consumption coagulopathy (14). The findings of thrombocytopenia, MAHA, and MODS involving vital organs tend to support the diagnosis of TTP-like syndrome, which should be included in the differential diagnosis of EVHD. Also, observed coagulation abnormalities, including hypofibrinogenemia, prolonged prothrombin time, activated partial thromboplastin time and fibrin degradation products (FDP), should alert hepatic coagulopathy (HC) as well as disseminated intravascular coagulation (DIC) and pathologic fibrinolysis (PF) in the differential diagnosis. This is particularly important since AH/AHNS is a significant component in EVD (1,12,15) and could be the cause of HC due to decreased hepatic synthesis of coagulation factors (15).

Proposed laboratory evaluation

First, to determine the nature of hemolysis, complete blood count, including hemoglobin, hematocrit, platelet count, reticulocyte count, haptoglobin, lactic acid dehydrogenase, Coombs' test, liver and renal

function tests, and review of peripheral blood films should be performed. An experienced hematologist in interpreting of blood cell morphology should examine blood films on several consecutive days to determine the presence of schistocytes. A demonstration of MAHA and unexplained thrombocytopenia with negative Coombs' test could support the diagnosis of TTP-like syndrome associated with EVD.

Second, to evaluate thrombotic microangiopathy and coagulopathy that may be associated with the hemorrhagic manifestation of EVHD, ADAMTS13 antigen and activity, ADAMTS13 antibody, VWF activity and multimeric pattern, prothrombin time, activated partial thromboplastin time, thrombin time, fibrinogen quantitation, fibrinolytic activity, FDP, and activities of factors VIII, V, VII, X and IX should be tested. Coagulation abnormalities, if present, may suggest the pathophysiologic mechanism underlies hemorrhagic manifestations.

In practice of real life, it is understood that some sophisticated hematologic and coagulation studies in evaluating suspected cases of TTP-like syndrome are unlikely to be available in West Africa and cannot be performed even in many hospitals of developed countries. For this reason, clinical research protocols should be designed to identify the pathophysiology of EVHD through proper blood collection at the patient care site and transport to a centralized laboratory facility to perform the needed tests. Once the pathophysiology and the diagnosis are confirmed, standard management regimens or clinical trials can be initiated.

Hematologic differential diagnosis

Both autoimmune TTP and TTP-like syndrome are thrombotic microangiopathies characterized by MAHA and thrombocytopenia. Compared with TTP, fewer schistocytes are common in certain TTP-like syndromes (11,16,17). In TTP-like syndromes associated with vascular injury following coronary artery bypass grafts and gastrointestinal surgeries, the presence of schistocytes may not be recognized on blood films unless approached with a high index of suspicion. If schistocytes are present in at more than 1–2% with confirmed hemolysis and thrombocytopenia, TTP-like syndrome should be strongly suspected (16,17). Unlike autoimmune TTP, in which ADAMTS13 activity is typically less than 5%, TTP-like syndrome shows moderately reduced ADAMTS13 activity at 20–70% (18). In both conditions, the activity of fibrinogen, and factors VII and V are always normal unless HC is present due to hypoxia in the liver. Without either AH/AHNS or severe thrombocytopenia, the bleeding should not occur in TTP-like syndrome because vascular microthrombi are solely made of aggregated platelets and ULVWF as bridges. EVHD typically has no bleeding tendency because coagulation factors are not involved in microthrombogenesis, and, instead, hypoxic organ dysfunction occurs. This may be the reason why the hemorrhagic manifestation of EVHD develops only in a minority of patients (1).

In acute DIC, fibrinogen is consumed and factors VIII and V are activated and then inactivated, but other coagulation factors participating in the tissue factor pathway are usually not consumed because inactivation does not take place following their activation. Thrombocytopenia in DIC develops due to consumption from blood clot formation (macrothrombi), but in thrombotic microangiopathy it occurs due to platelet thrombus formation (microthrombi). In acute DIC, hemorrhagic manifestation is the predominant feature and thrombotic manifestation is uncommon. The bleeding from orifices tends to form less firm clots, perhaps due to insufficient amounts of factors VIII and V, and fibrinogen, and secondary fibrinolysis. During a minor vascular access such as routine venipuncture, uncontrollable bleeding frequently occurs.

In postoperative TTP-like syndrome, vascular microthrombosis is more likely to occur in the brain, liver and lungs, resulting in CNSD, AH/AHNS and ARDS (11,16). If the disease progresses to MODS and SIRS, AH/AHNS is likely to occur and HC associated with hypofibrinogenemia, prolonged prothrombin time, activated partial thromboplastin time, and elevated FDP in the presence of thrombocytopenia could mimic DIC. In this case, excessive bleeding tendency could be erroneously blamed on DIC without the confirmation of laboratory diagnosis. Although establishing the diagnosis of DIC is a real challenge, it should be clearly differentiated from HC. In HC, factor VIII activity is normal or elevated, but the activity of factors VII, V, X and IX is decreased. The differential diagnosis of various coagulopathies is summarized in Table 1. In the literature, MAHA occurring in DIC is often mentioned (19). This claim should be carefully evaluated since the possibility of TTP-like syndrome associated with HC cannot be excluded. Proper hematologic evaluation should resolve this issue.

Potential treatment consideration

For EVD, development of therapeutic antiviral agents should be vigorously pursued. If EVHD is confirmed to be TTP-like syndrome, daily

TABLE 1. *Differential diagnosis and characteristics of coagulopathies*

	TTP-like syndrome	HC due to AHNS	Acute DIC	PF
Thrombocytopenia	Always present	Always present	Always present	Not present
MAHA	Typically always present	Usually present	May be present	Not present
Fibrinogen	Normal	Usually decreased	Always decreased	Always decreased
Factor VIII	Normal	Normal or increased	Markedly decreased	Decreased
Factor V	Normal	Decreased	Decreased	Decreased
Factor X	Normal	Decreased	Usually normal	Normal
Factor VII	Normal	Markedly decreased	Normal	Normal
Factor IX	Normal	Decreased	Normal	Normal
FDP	Normal	Positive	Strongly positive	Strongly positive
Thrombin time	Normal	Prolonged	Prolonged	Prolonged
Thrombosis form	Microthrombi	Microthrombi	Friable macrothrombi	Absent
Bleeding: Character	Rare, mild petechiae[†]	May cause serious bleeding	Common, serious bleeding	Slow & persistent bleeding
Treatment	Usually no need of treatment[†]	Controllable with FFP	Uncontrollable & not effective	Treatable with AFA
Platelet transfusion	Usually contraindicated	Consider if platelets <12 000/μL	Probably not effective	No need

AFA, anti-fibrinolytic agents; AHNS, acute hepatic necrosis syndrome secondary to TTP-like syndrome; DIC, disseminated intravascular coagulation; FDP, fibrin degradation products; FFP, fresh frozen plasma; HC, hepatic coagulopathy; MAHA, microangiopathic hemolytic anemia; PF, pathologic fibrinolysis; TTP, thrombotic thrombocytopenic purpura. [†]Unless platelet count is less than 12,000/μL

therapeutic plasma exchange (TPE) could be considered as a therapeutic intervention to reverse the pathologic microthrombotic process. Although the potential benefit of TPE in EVD-associated TTP-like syndrome is not well characterized, it may correct ADAMTS13 insufficiency as seen in certain TTP-like syndromes (20) and perhaps contribute to the clearance of inflammatory cytokines. In certain situations, TPE may replace the enzyme pool with a catalytically more efficient ADAMTS13. TPE has shown a better outcome on sepsis-associated SIRS and MODS in previously healthy pediatric patients if instituted early in the course of the disease (21). An improvement of CNSD, ARDS, AS/AHNS as well as thrombocytopenia, MAHA, hypoxemia, and hypertransaminasemia would suggest early clinical and hematologic response to TPE, which usually begins to occur within 2–4 days if diagnosed and treated in a timely manner (9,11,16). However, the benefit is limited if MOF is too advanced.

The Centers for Disease Control and Prevention (CDC) has made specific recommendations for safely performing acute hemodialysis in patients with EVD in US hospitals (22). If TTP-like syndrome is confirmed, this guideline can be applied to TPE. However, in many clinical settings, this treatment might be impractical due to a potential risk of viral contamination to healthcare workers and facilities as well as to medical equipment. Additionally, worldwide use of TPE may not be economically viable. In that situation, fresh frozen plasma (FFP) therapy can be considered (13). Yet, pooled FFP is another alternative treatment. FFP or pooled FFP would be also the treatment of choice for the bleeding due to HC associated with AH/AHNS.

Although passive antibody therapy from recovered EVD patients has been used, conflicting evidence exists regarding the efficacy of convalescent plasma and whole blood (23). Other potential pharmacologic options, including recombinant ADAMTS13 (24), anti-VWF aptamers, anti-VWF nanobody, and N-acetyl cysteine, should be explored. These biological agents could become effective anti-microthrombotic drugs in the future.

In conclusion, EVHD should be carefully evaluated with appropriate hematologic tests to confirm or refute the potential diagnosis of TTP-like syndrome. If the diagnosis is well established, a new approach in the management of EVHD could begin to complement optimal supportive care. Little can be said about the potential impact of TPE on possible EVD-associated TTP-like syndrome at this time. Further reports and studies would provide additional evidence to better characterize the use of plasma and TPE in treating this specific thrombotic microangiopathy.

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REFERENCES

1. Fowler RA, Fletcher T, Fischer, II WA et al. Caring for critically ill patients with Ebola viral disease: perspectives from West Africa. *Am J Respir Crit Care Med* 2014;190:733–7.
2. Sullivan N, Yang ZY, Nabel GJ. Ebola virus pathogenesis: implications for vaccines and therapies. *J Virol* 2003;77:9733–7.
3. Feldmann H, Volchkov VE, Volchkova VA, Klenk HD. The glycoproteins of Marburg and Ebola virus and their potential roles in pathogenesis. *Arch Virol Suppl* 1999;15:159–69.
4. Wahl-Jensen VM, Afanasieva TA, Seebach J, Stroeher U, Feldmann H, Schnittler HJ. Effects of Ebola virus glycoproteins on endothelial cell activation and barrier function. *J Virol* 2005;79:10442–50.
5. Riabchikova EI, Kolesnikova LV, Rassadkin IuN. Microscopic study of species specific features of hemostatic impairment in Ebola virus infected monkeys. *Vestn Ross Akad Med Nauk* 1998;3:51–5.
6. Kraisin S, Naka I, Patarapotikul J et al. Association of ADAMTS13 polymorphism with cerebral malaria. *Malar J* 2011;10:366.
7. Larkin D, de Laat B, Jenkins PV et al. Severe Plasmodium falciparum malaria is associated with circulating ultra-large von Willebrand multimers and ADAMTS13 inhibition. *PLoS Pathog* 2009;5:e1000349.
8. Tsai HM. Mechanisms of microvascular thrombosis in thrombotic thrombocytopenic purpura. *Kidney Int Suppl* 2009;(112):S11–4.
9. Chang JC, Aly ES. Acute respiratory distress syndrome as a major clinical manifestation of thrombotic thrombocytopenic purpura. *Am J Med Sci* 2001;321:124–8.
10. Kathula SK, Koduri J, Mall S, Chang JC. A case report of total abdominal hysterectomy resulting in acute thrombotic thrombocytopenic purpura with pancreatitis and hepatitis: complete resolution with plasma exchange therapy. *Ther Apher Dial* 2003;7:373–4.
11. Chang JC, Kathula SK. Various clinical manifestations in patients with thrombotic microangiopathy. *J Investig Med* 2002;50:201–6.
12. Kortepeter, MG, Bausch, DG, Bray, M. Basic Clinical and Laboratory Features of Filoviral Hemorrhagic Fever. *J Infect Dis* 2011;204(Suppl 3):S810–6.
13. Hukkanen RR, Liggitt HD, Murnane RD, Frevert CW. Systemic inflammatory response syndrome in nonhuman primates culminating in multiple organ failure, acute lung injury, and disseminated intravascular coagulation. *Toxicol Pathol* 2009;37:799–804.
14. Fisher-Hoch SP, Platt GS, Lloyd G, Simpson DI, Neild GH, Barrett AJ. Haematological and biochemical monitoring of Ebola infection in rhesus monkeys: implications for patient management. *Lancet* 1983;2:1055–8.
15. Paessler S, Walker DH. Pathogenesis of the viral hemorrhagic fevers. *Annu Rev Pathol* 2013;8:411–40.
16. Chang JC, Shipstone A, Llenado-Lee MA. Postoperative thrombotic thrombocytopenic purpura following cardiovascular surgeries. *Am J Hematol* 1996;53:11–17.
17. Chang JC, Newman RS. Redefining the syndromes of thrombotic microangiopathy. *Ther Apher Dial* 2004;8:73–4.
18. Saltzman DJ, Chang JC, Jimenez JC et al. Postoperative thrombotic thrombocytopenic purpura after open heart operations. *Ann Thorac Surg* 2010;89:119–23.
19. Jacobson RJ, Jackson DP. Erythrocyte fragmentation in defibrination syndromes. *Ann Intern Med* 1974;81:207–9.
20. Nguyen TC1, Han YY, Kiss JE et al. Intensive plasma exchange increases a disintegrin and metalloprotease with thrombospondin motifs-13 activity and reverses organ dysfunction in children with thrombocytopenia-associated multiple organ failure. *Crit Care Med* 2008;36:2878–87.
21. Qu L, Kiss JE, Dargo G, Carcillo JA. Outcomes of previously healthy pediatric patients with fulminant sepsis-induced multisystem organ failure receiving therapeutic plasma exchange. *J Clin Apher* 2011;26:208-13.
22. http://www.cdc.gov/vhf/ebola/hcp/guidance-dialysis.html
23. Mupapa K, Massamba M, Kibadi K et al. Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. International Scientific and Technical Committee. *J Infect Dis* 1999;179(Suppl 1):S18–23.
24. Plaimauer B, Kremer Hovinga JA, Juno C et al. Recombinant ADAMTS13 normalizes von Willebrand factor-cleaving activity in plasma of acquired TTP patients by overriding inhibitory antibodies. *J Thromb Haemost* 2011;9:936–44.