

Haematological Disturbances in Dengue Haemorrhagic Fever - Its Pathogenesis and Management Perspectives

Kolitha H Sellahewa^{1*}

1.Dr Neville Fernando Teaching Hospital, Millenium Drive, Malabe, Sri Lanka

Abstract

Haemorrhage is common to both dengue fever (DF) and dengue haemorrhagic fever (DHF). Thrombocytopenia is exceedingly common, while prolonged partial thromboplastin time and reduced fibrinogen concentration are the other abnormal haemostatic indices evident from early in the disease course. These haematological abnormalities correlate better with the timing and severity of plasma leakage rather than the clinical haemorrhagic manifestations. Blood products including prophylactic platelet transfusions are hardly required in the management of DHF. Judicious fluid therapy is the most effective intervention to prevent complications and bleeding in DHF. Concealed haemorrhage is an important complication requiring early recognition and blood transfusions to improve outcomes. Understanding the pathogenesis of coagulopathy and the significance of altered haemostatic indices, and its co-relation to disease severity and phase of DHF, is essential for appropriate interventions particularly when DHF co-exists in patients on mandatory anticoagulation for prosthetic heart valves and atrial fibrillation.

Corresponding author:

Kolitha H. Sellahewa, Dr Neville Fernando Teaching Hospital, Millenium Drive, Malabe, Sri Lanka

email: kolithah@gmail.com, mobile: + 94 0718433213

Citation: Kolitha H Sellahewa (2015) Haematological Disturbances in Dengue Haemorrhagic Fever - its Pathogenesis and Management Perspectives. Journal of Hematology and Oncology Research - 1(3):15-24. <https://doi.org/10.14302/issn.2372-6601.jhor-14-381>

Key words: Dengue haemorrhagic fever, haematological disturbances, management.

Received : Dec 23, 2013

Accepted : Aug 21, 2014

Published : Jan 23, 2015

Introduction

Infection by any one of the four serotypes of dengue virus (DENV) remains asymptomatic in the vast majority. Clinical spectrum among symptomatic infection ranges from undifferentiated fever (viral syndrome), DF, and DHF to the expanded dengue syndrome with isolated organopathy (unusual manifestations). The commonest clinical types are DF and DHF and bleeding manifestations are common to both. Severe disease including shock is exclusive to DHF, which has plasma leakage and abnormal haemostasis as its pathological hall marks. The WHO criteria for the clinical diagnosis of DHF requires the presence of acute and continuous fever of 2 to 7 days, haemorrhagic manifestations associated with thrombocytopenia (10×10^9 /L or less) and haemoconcentration (haematocrit >20% from baseline of patient or population of same age). Haemorrhagic manifestations could be mucosal and or skin or even a positive tourniquet test which is the commonest. DF can be without haemorrhage or have unusual haemorrhage (1). The main factor implicated in the development of DHF rather than the relatively innocuous DF in dengue infection is secondary dengue infection but other factors like viral virulence, and host characteristics are also important. Severe disease is the result of a complex interaction between the virus and the immune response evoked by the host with secondary infection (2).

In both DF and DHF clinical bleeding is often mild and manifest as petechial haemorrhages, ecchymoses, gum

bleeding or merely a positive tourniquet test. Severe disease can cause severe mucosal bleeding manifesting as hematemesis, melena and menorrhagia or concealed haemorrhage to many organs. Haematological disturbances prevail in the majority though co-relating poorly with clinical bleeding. These range from thrombocytopenia, prolonged prothrombin time, partial thromboplastin time, low plasma fibrinogen and disseminated intravascular coagulation.

Pathogenesis

Mechanisms of haemorrhage in dengue are complex and poorly understood. However well recognised coagulation disturbances do exist. Thrombocytopenia is a consistent finding, while prolonged partial thromboplastin time and reduced fibrinogen concentration are the other abnormal haemostatic indices evident from early in the disease course. These haematological abnormalities seem to correlate better with the timing and severity of plasma leakage rather than the clinical haemorrhagic manifestations (3).

Thrombocytopenia is initially due to bone marrow suppression during the febrile viraemic phase of the illness. Progressive thrombocytopenia with defervescence result from immune mediated platelet destruction. Virus-antibody complexes have been detected on the platelet surface of DHF patients suggesting a role for immune-mediated destruction of platelets (4, 5). Augmented platelet adhesiveness to vascular endothelial cells resulting from the release of high levels of platelet-activating factor by monocytes

with heterologous secondary infection also contributes to the thrombocytopaenia (6). Thrombocytopaenia however correlates poorly with bleeding manifestations (7). Spontaneous bleeding is uncommon even with counts below 10×10^9 /L. It is strongly associated with the severity of vascular leakage. Counts below 10×10^9 /L or a rapid drop in the platelet count was associated with severe disease.

The role of the glycocalyx rather than the endothelial cells per se in controlling ultrafiltration in the microvasculature is increasingly recognised and in vivo animal studies have shown the permeation of fibrinogen to the endothelial surface similar to albumin (8). The low plasma fibrinogen detected in DHF could thus be a reflection of loss into the interstitial spaces in the setting of increased vascular permeability. Heparan sulphate forms an integral part of the glycocalyx which when damaged by the initial cytokine response in DHF gets liberated to the circulation and acts like an anticoagulant which could explain the prolonged activated partial thromboplastin time (APTT) (9). The disturbance in both these important haemostatic indices are unlikely to cause spontaneous bleeding. Haemorrhages are triggered by trauma in this setting of coagulopathy. These recent findings raise the possibility for common pathogenic mechanisms responsible for both plasma leakage and abnormalities in the haemostatic indices. The true nature of the intrinsic coagulopathy evident early in the disease course and in mild forms of dengue can be confounded by the advent of hypovolemic shock

and hypoxia in DHF with severe plasma leakage with less than optimal correction. Thus low plasma fibrinogen and prolonged APTT in the absence of shock early in the disease is to be expected in DHF and interpreted as heralding plasma leakage and not disseminated intravascular haemorrhage (DIC), and its magnitude gives an idea of the severity of leakage. On the contrary the same indices of coagulopathy should have a different interpretation in the setting of shock owing to the confounding effects of hypovolemia and hypoxia and even the probability of associated DIC in such a setting (3).

Development of antibodies potentially cross-reactive to plasminogen could have a role in causing haemorrhage in DHF (10). However different studies have shown conflicting results as some have demonstrated an activation of fibrinolysis while others have shown an inhibition of the fibrinolytic pathway in DHF (4). The low levels of thrombin-activatable fibrinolysis inhibitor (TAFI) demonstrated in patients with severe DHF could result in an impairment of clot stabilization. The resultant imbalance between coagulation and fibrinolysis is another contributor to haemorrhage (11).

Endothelial cells in DHF play an important role in the pathogenesis of coagulopathy in DHF. However precise knowledge on the extent to which DENV infects endothelial cells is lacking as few studies have addressed the issue in the viraemic phase of the illness. Even though DENV has infected endothelial cells in vitro it is doubtful whether it reflects the effect in human infection

as limited human autopsy studies have detected only the dengue antigen but not the genome in various cell types ranging from monocytes, liver sinusoidal cells, alveolar macrophages, peripheral blood, and splenic lymphocytes. How important these findings are in the pathogenesis of clinical features are uncertain as some studies have shown swelling of endothelial cells but not cell death or vasculitis (12), while others have detected apoptosis of endothelial cells in lungs and intestinal mucosa in fatal DHF cases, but the extent of apoptosis has not been documented (12). DENV alters the endothelial cell surface protein production, its expression, and transcriptional activity. The role of DENV infected endothelial cells in the pathogenesis of coagulopathy in DHF is intriguing. There is up regulation of tissue plasminogen, thrombomodulin, protease activated receptor-1, and tissue factor receptor, while there is down regulation of tissue factor inhibitor and activated protein C.

The recent observation of an imbalance in the vWF-ADAMTS-13 system in dengue infection adds further complexity to the pathogenesis of coagulopathy. Von Willibrand factor (vWF) is synthesised mainly in the vascular endothelium. After cleavage of a vWF propeptide it is stored in specialised secretory granules called Weibel-Palade bodies (WPBs). Endothelial injury or activation releases vWF which facilitates platelet adhesion, aggregation and generation of platelet rich microthrombi owing to the prothrombogenic effect of the freshly released vWF in the form of ultra large

multimers (UL-vWF). The metallo-protease ADAMTS-13 deactivates the prothrombogenic UL-vWF and acts as a natural regulator. The recent discovery of low levels of ADAMTS-13 and high levels of vWF in an elongated active conformation in patients with severe DHF adds another pathway to explain the thrombocytopenia and its co-relation to disease severity. The elongated conformation of vWF exposes the platelet glycoprotein receptor Ib facilitating platelet aggregation and the generated platelet rich microthrombi would clog the microcirculation and aggravate tissue hypoxia and organ dysfunction. The association of severe disease with blood group AB is also explicable on the role of vWF in dengue pathogenesis as blood group AB has higher vWF levels (13).

Hemophagocytic lymphohistiocytosis is a rare haematological disturbance reported in some patients with severe DHF which can contribute to thrombocytopenia and progressive haemolysis. It results from a hyperimmune interaction of macrophages with dengue virus (14).

Leucopenia is consistently seen in the viraemic phase owing to bone marrow suppression and granulocytosis is unusual and its presence should alert the clinician to suspect the possibilities of concealed haemorrhage, a response to continuing haemolysis, or co-infection.

In summary complex mechanisms result in an imbalance between coagulation and fibrinolysis predisposing to haemorrhage on the one hand and microthrombi clogging up the microvasculature and leading to Multiple

organ dysfunction (MODS) on the other hand. The clinically important disturbances in haemostatic indices seen in dengue infection ranges from leucopaenia, thrombocytopaenia, hypofibrinogenaemia, prolonged PT and APTT to granulocytosis. Bone marrow suppression in the initial viraemic phase of the illness, immune mediated destruction, and altered function of haematological elements and coagulation factors, permeation of clotting factors with increased vascular leakage, destruction of the glycocalyx and release of heparan sulphate and altered endothelial function all play a role in the expression of haemostatic disturbances. The extent to which each one of these mechanisms are implicated in the pathogenesis depends on the phase and severity of the disease. This is an important notion to bear in mind in the interpretation of abnormal haemostatic indices in making accurate and appropriate management decisions on intervention.

Management perspectives

Haemostatic disturbances occur in both DF and DHF from early in the disease course but do not directly correlate with clinical bleeding. It is important to keep this in mind to avoid inappropriate, albeit harmful interventions based on the mere detection of altered haemostatic indices.

Preventive strategies:

Management priority should be to prevent more serious and significant concealed or overt haemorrhage stemming from acidosis and hypoxia consequent to less than optimal fluid therapy and attended haemodynamic

compromise. Acidosis is a major contributor to serious life threatening haemorrhage. Hence it should be corrected early in anticipation of severe bleeding later in the course of the disease in any patient with DHF developing shock. Intervention with intravenous sodium bicarbonate is for a pH of 7.35 or less. The cut off pH is higher than that used for shock from other disorders like sepsis owing to the critical role of acidosis in the amplification of haemostatic disturbances in DHF leading to significant clinical bleeding. If blood gas facilities are not available empirical use of sodium bicarbonate is justifiable under these circumstances (1, 15).

Platelet transfusions:

Thrombocytopaenia is a consistent finding but does not require routine prophylactic platelet transfusions. This has been my personal experience with patients managed over the last 15 years (unpublished data) which has been supported by recently published data (16). Prophylactic platelet transfusions had not shown a significant difference in occurrence of haemorrhage when compared to those who had not received platelets among paediatric patients with dengue shock syndrome (17, 18). Platelet transfusions can cause allergic reactions, alloimmunisation, febrile non haemolytic reactions, bacterial, viral and parasitic infections, acute lung injury and pulmonary oedema from fluid overloading in patients with DHF (17, 18, 19,). Platelet transfusions even with platelet counts as low as $5-10 \times 10^9/L$ per se is not indicated for stable patients even in the presence of minor bleeding without compromise of

patient safety and favourable outcomes. Provided proper fluid management has ensured haemodynamic stability and there are no additional risk factors for bleeding such as prolonged fever, sepsis and significant coagulopathy. It is indicated for significant bleeding i.e. >10ml/kg body weight in association with a platelet count of $<10 \times 10^9/L$, DIC, and in patients with intracranial haemorrhages. Platelet transfusions should also be given prophylactically immediately prior to emergency caesarean section or any other urgent surgery (15).

Blood transfusions:

Blood transfusions should be given early rather than late to minimise tissue hypoxia and attended adverse complications like MODS. A drop in the haematocrit associated with haemodynamic instability despite fluid resuscitation should be a trigger for blood transfusion owing to the possibility of concealed haemorrhage. The presence of a polymorphonuclear leucocytosis in the absence of sepsis in this clinical setting tends to support this contention. Either whole blood or packed red cells could be given depending on the volume status and phase of illness (1, 15).

Haematocrit:

All patients with dengue should have a base line full blood count and haematocrit (HCT). Frequent estimations of haematocrit during the critical phase of 24-48 hours among DHF patients are essential for the early detection of plasma leakage and determine the adequacy of fluid therapy. A 20% increase in the haematocrit reflects haemoconcentration from plasma

leakage. Co-relating the temporal changes in HCT with vital signs should be used to adjust fluid infusion rates to match the dynamics of plasma leakage. Haematocrit is also useful in determining the adequacy of fluid intervention for patients in shock. Thus HCT should drop by 10 points after a bolus of Dextran 40. On the contrary a drop in the HCT after fluid resuscitation with no improvement in haemodynamic status reflects concealed haemorrhage (15). HCT is also a vital haematological parameter to detect fluid overload both in the critical and convalescent phases of DHF. Fluid overloading and the attended haemodilution would be reflected as a low HCT. Its diagnostic and therapeutic utility for accurate and appropriate selection of the quantity and quality (crystalloid, colloid or blood) of fluid requires intelligent co-relation of its kinetics to the phase of the illness.

Fresh frozen plasma:

Prophylactic transfusions of fresh frozen plasma (FFP) are also not usually indicated owing to the risk of fluid overload, anaphylactic reactions and transfusion related infections. However it may be beneficial for patients with hepatic encephalopathy with active bleeding (15). Replenishment of clotting factors could normalise coagulopathy. An added benefit of FFP is its effect on the platelet count. Immunomodulating effects could block immune mediated platelet destruction and replenishment of ADAMTS-13 in concert could increase the platelet count with attended benefit in minimising bleeding (13, 20). Use of FFP as an intervention to

reduce plasma leakage is also hypothesised (21). Use of FFP in DHF should be an individualised decision based on astute clinical judgement that should take into consideration the phase of the illness, volume status, bleeding tendency, presence or absence of DIC and hepatic encephalopathy and carefully weighing the pharmacological benefits of such an intervention against the known risks.

Recombinant factor VII:

There is no evidence to support the use of recombinant factor VII in DHF patients with bleeding due to prolonged shock DIC or MODS. Its use for bleeding in DHF due to such conditions is not recommended. Recombinant factor VII is useful only in patients who have massive bleeding due to a specific cause such as bleeding oesophageal Varices, peptic ulcer or bleeding from an identifiable site in the nose prior to planned surgical intervention. This helps to buy time for the specific surgical treatment like banding, cauterization etc. It should be used only if definite plans are there for surgical intervention as the arrest of bleeding with recombinant factor VII is only temporary (15).

Special situations:

The increasing trend of dengue infection worldwide and the large number of patients on mandatory anticoagulation owing to co-morbid states like atrial fibrillation and prosthetic heart valves increases the likelihood of dengue infection co-existing among patients on mandatory anticoagulation. Such an event could pose

a challenging management dilemma to the attending clinician, of whether to continue anticoagulation in the setting of a disease with disordered haemostasis and thereby augment the risk of haemorrhage; or discontinue anticoagulation and risk fatal valve thrombosis or a crippling thromboembolic event as the case may be. Apart for one case report neither clear guidelines nor clinical experience is available regarding the best management strategy for such clinical situations (22). Taking into consideration the well-recognised coagulation disturbances and its pathogenesis; my advocacy would be to stop warfarin early in the disease course owing to the long half-life of warfarin and the prolonged PT and APTT imposed by the haemostatic disturbance of DHF. Tendency for valve thrombosis is further reduced despite stopping warfarin owing to the intrinsic anticoagulatory effect from heparan sulphate released from the damaged endothelial glycocalyx. As the haemorrhagic risk is minimal with good fluid management, and the greatest risk of haemorrhage is in the critical phase of DHF, as well as the transitory nature the coagulatory derangement, warfarin could safely be recommenced towards late critical or early convalescent phase so that sufficient time is given to establish warfarin effect. Frequent monitoring of the PT and APTT and its correlation to the disease phase and conceived clearance of warfarin based on knowledge of its pharmacokinetics, rather than dynamics of the platelet count alone would be the most appropriate haemostatic indices to guide

timing of both cessation and recommencement of warfarin therapy.

Dengue infection in patients with sickle cell anaemia (SCA) or sickle cell disease (SCD) is another special situation that could be challenging to the clinician as two cases of fatality have been reported (23). SCD and SCA damage the vascular endothelium and the attended contribution to coagulopathy and vascular leakage increases the propensity to develop shock and MODS. This tendency must be borne in mind when managing DHF among patients with SCD and SCA and may necessitate a more aggressive approach to fluid therapy.

Concluding remarks

Complex pathogenic mechanisms in both coagulatory and fibrinolytic pathways are implicated in the haematological disturbances in DHF. The altered haemostatic indices however, co-relate poorly with clinical bleeding and consequently blood products including prophylactic platelet transfusions are hardly required in the management of DHF. The risk of allergic reactions and transfusion related infections far outweigh any benefit in preventing significant haemorrhage from inappropriate replacement therapy. Judicious fluid therapy is the most effective intervention to prevent complications and bleeding in DHF. Concealed haemorrhage is an important complication requiring early recognition and blood transfusions to improve outcomes.

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