

Dengue Hemorrhagic Fever: Disorders of Hemostasis

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Dengue illness caused by any of the four serotypes of dengue viruses (DEN1-4) is currently the most important mosquito-borne viral disease in the tropical areas of the world. It has been estimated that 20 million cases of dengue occur annually worldwide; around 500,000 cases, mainly the most severe form, dengue hemorrhagic fever (DHF), require hospitalization. With the case fatality rate of DHF varying from 1% to 5% a significant number of deaths, around 24,000 cases, occur each year, most of them are children⁽¹⁾.

Dengue virus infections can be asymptomatic or cause any of the following illnesses, undifferentiated fever, dengue fever (DF) and DHF. Dengue fever is a syndrome of 5-7 days of fever, headache, myalgia, bone/joint pain and rash, often accompanied by leukopenia. Occasionally variable degrees of thrombocytopenia and hemorrhage (usually cutaneous) are observed. More severe cases with incapacitating bone/joint pain ("break-bone fever") are common among adults. Infrequently DF may be accompanied by unusual bleeding complications that may cause death⁽¹⁾. DHF which was recognized in the 1950s, occurs almost exclusively in children and is associated with high mortality.

Clinical features of DHF resemble those of DF in early febrile phase in many respects. The prominent feature of DHF is its potential to develop into fatal dengue shock syndrome (DSS). The major pathophysiologic hallmarks that determine disease severity and distinguish DHF from DF and other viral hemorrhagic fevers are plasma leakage due to increased vascular permeability and abnormal hemostasis⁽¹⁸⁾. Hypovolemic shock occurs as a consequence of, and subsequent to, critical plasma volume loss. Abnormal hemostasis including increased capillary fragility (positive tourniquet test and easy bruising at the site of venepuncture), thrombocytopenia, impaired platelet function and consumptive coagulopathy in the most severe form disseminated intravascular coagulation (DIC), contribute to varying degrees of hemorrhagic manifestations^(1,11,16,20).

Over the past four decades extensive studies in various fields have suggested that DHF is induced by immunopathological mechanisms that involve both humoral and cell-mediated immune responses^(1,9,13). Complement activation with profound depression of C3 and C5 levels are constant findings and thought to play important role in the pathogenesis of DHF/DSS^(5,23). The association of DHF/DSS with secondary dengue infection, with dengue virus different from the first (primary) infection, lead Halstead to study and describe antibody dependent enhancement (ADE) of dengue virus infection in macrophage or mononuclear cells.⁽⁹⁾ It is proposed that an increase in the number of dengue virus-infected monocytes due to enhancing activity of the cross-reacting antibody from previous infection was responsible

for the pathogenesis of DHF. Kurane and Ennis proposed that in addition to ADE and activation of complements, activation of lymphocytes also play an important role⁽¹³⁾. Activation of monocytes and T cells induce the production of cytokines and chemical mediators. They hypothesize that a rapid increase in the levels of the potential mediators such as TNF α , IL-2, IL-6, IFN- γ , PAF, C3a, C5a and histamine and the synergistic effects of these mediators induce malfunction of vascular endothelial cells, which leads to plasma leakage, shock, and derangements of the coagulation system, which may lead to hemorrhagic manifestations^(12,13).

At present the mediators and the precise mechanism(s) of plasma leakage and bleeding phenomena in DHF have not yet been identified, and more studies are needed. Nonetheless, the results of various studies, particularly those of clinical and pathophysiologic changes, have contributed a great deal to improvement in management of the patients and a marked reduction in the mortality rate. Among various fields, hemostatic disorders have been widely studied, the results of which are briefly reviewed in this communication.

Clinical Course of DHF

DHF is typically characterized by four major clinical manifestations presented in order of appearance and frequency, as follows:^(1,18)

- High continuous fever for 2 to 7 days in most cases
- Hemorrhagic manifestations: a positive tourniquet test, petechiae, epistaxis, and gastrointestinal bleedings
- Hepatomegaly
- Circulatory disturbances (shock in severe cases)

Thrombocytopenia and hemoconcentration (rising hematocrit from base line of 20% or more) representing the pathophysiologic hallmarks of abnormal hemostasis and plasma leakage, respectively, are constant laboratory findings. These changes occur simultaneously before subsidence of fever and before onset of shock. Therefore, they are important for clinical diagnosis.

The illness typically begins abruptly with high fever accompanied by facial flushing, skin erythema, headache, anorexia, vomiting and other symptoms similar to those of DF. The positive tourniquet test and the presence of skin petechiae are observed early in the febrile phase. Other hemorrhagic manifestations observed during the febrile phase include easy bruising at the site of venipuncture, epistaxis, and gum bleeding, which are usually mild. Gastrointestinal bleeding as hematemesis and melena are rarely observed during the febrile phase.

Liver enlargement is observed on day 3-4 of illness in

over 90% of cases in children and around 60% of adults. Splenomegaly is rarely observed in infants⁽¹⁾.

The critical stage of DHF is reached by the end of the febrile period, after 2–7 days of fever, when plasma leakage commences. The period of plasma leakage is about 24–48 hours. Patients may complain of abdominal pain, and there may be mild and transient changes in pulse rate and blood pressure when the temperature drops to normal. In severe cases, following critical plasma volume loss, when the temperature drops shock ensues and progresses rapidly to profound shock and death if proper treatment is not given. The period of shock is short but life threatening, but the patient who receives proper treatment has a rapid and uneventful recovery. In case of prolonged shock, often with metabolic acidosis, severe bleeding, usually gastrointestinal, in the form of hematemesis and melena may occur and confers a poor prognosis with a high mortality rate.

In most cases, early and effective replacement of lost plasma with a glucose electrolyte solution, plasma or plasma expanders results in a favorable outcome. Blood transfusion is indicated in patients with severe bleeding. Convalescence is short and uneventful. Confluent petechial rashes on extremities may be observed after defervescence. The duration of an uncomplicated case of DHF/DSS is about 7 to 10 days⁽¹⁾.

Pathophysiologic and Pathologic Changes

The most prominent feature of DHF is plasma leakage, which appears to be selective, into the pleural and abdominal cavities^(1,18). Pericardial effusion, if any, is rather minimal. Other evidence of plasma leakage includes a rising hematocrit, hypoproteinemia, and hypoalbuminemia. Although the pathogenesis of plasma leakage is not yet well identified, clinical observation and pathologic studies suggest that the increase in vascular permeability leading to plasma leakage and shock is most likely a functional change due to short acting mediators, products of the immune response, rather than to structural destruction of endothelial cells. Evidence for this includes:

- Rapid onset of plasma leakage with sudden elevation of hematocrit
- Short duration of shock and plasma leakage for 24 to 48 hours
- Rapid recovery with proper treatment (24 to 48 hours) with uneventful convalescence
- No sequelae
- No inflammatory vascular changes found at autopsy⁽²⁾
- No severe pathologic changes in major organs other than serous effusion and hemorrhage⁽²⁾

Two observations are important to understand the mechanisms of leakage and shock. One is that the DHF patient has no generalized edema when presenting with shock. This is in support of a selective leakage into serous spaces⁽¹⁸⁾. The second is that the timing of leakage is from the end of the febrile phase into 24–48 hours after defervescence, which follows from the earlier period during which

the tourniquet test becomes positive and the petechiae first appear. These observations suggest that vascular change (vasculopathy) of increased vascular permeability that leads to plasma leakage probably occurs in venules⁽³⁾ in the thorax and abdominal cavities, while those changes related to hemorrhage in the form of diapedesis of erythrocytes is probably conformed to capillaries⁽³⁾.

Among possible mediators, it has recently been shown that C3a and C5a are elevated and that both the levels and duration of elevation correlated well with the occurrence of shock and disease severity⁽¹⁴⁾. Among the cytokines and chemical mediators, including tumor necrosis factor, interleukin-1 (IL-1), IL2, IL6, IFN- γ , all except IL-1 have been found to be elevated in DHF⁽¹³⁾. Although the studies suggest an important effect of these cytokines and chemical mediators(s), they are still far from being conclusive. Further studies in search of the mediators responsible for plasma leakage are necessary and a top priority.

Hemorrhage and Hemostatic Disorders

Hemorrhagic manifestations, which are invariably present in DHF, are usually mild and most commonly found as scattered tiny petechiae in the skin and occasionally submucosa. A positive tourniquet test, which indicates increased capillary fragility, is the most common finding that appears early. Massive bleeding that requires blood transfusion is less common and usually occurs after onset of shock⁽¹⁸⁾. Gastrointestinal bleeding in the form of hematemesis and/or melena are the most common severe bleeding symptoms. In those who die after prolonged shock, bleeding in various organs, such as the gastrointestinal tract, heart, lungs, liver, and brain have been observed^(2,19). Some preexisting host factors may contribute to gastric bleeding (hematemesis), early in the course of illness. Examples of this phenomenon are hematemesis due to pre-existing peptic ulcer or gastritis by aspirin ingestion⁽¹⁰⁾.

Peripheral Blood and Bone Marrow

Leukopenia is a common finding both in DF and DHF⁽¹⁰⁾. The leukocyte counts may be normal or slightly increased with predominant by neutrophils initially. Towards the end of the febrile phase there is a reduction in the number of total leukocytes and neutrophils. Simultaneously relative lymphocytosis is noted with the presence of atypical lymphocytes. The leukopenia usually reaches its nadir shortly before or at the time of a drop in temperature and returns to normal 2–3 days after defervescence. The number of atypical lymphocytes in DHF are clearly greater than in patients with DF. These cells may represent blast transformation of activated B and T lymphocytes^(7,13). Reduction in the number of platelets usually follows that of leukocytes and reaches the nadir on the day of defervescence. Giant platelets have been observed in blood smears of DHF patients, suggesting an increased platelet turnover⁽³⁾.

The bone marrow changes are similar in DF and DHF^(4,7,10). During the febrile phase there is hypocellularity with arrest of the maturation of all elements, particularly

megakaryocytes. A total absence of granulocytopoiesis was observed in a case of DHF studied on day 4 of the illness⁽⁴⁾. These data suggest that the decrease of neutrophils observed during the febrile period of dengue infection may be due to destruction or inhibition of myeloid progenitor cells and maturation arrest. During the time of shock when the platelet count reaches a nadir, the bone marrow showed normal- or hypercellularity; megakaryocytes became normal and sometimes increased in numbers. During convalescence there is a rapid recovery of all marrow elements⁽¹⁷⁾. The mechanism of transient bone marrow suppression in dengue is unknown. It could be a direct effect of the virus or an indirect effect through immune mechanisms, or both.

Platelets and Platelet Function

Thrombocytopenia is a constant finding in DHF/DSS. The platelet counts usually drops to below 100,000/mm³ 1–2 days before defervescence and remains low for 3–5 days in most cases. The levels then increase rapidly to normal during convalescence. The platelet counts in shock cases are frequently below 50,000/mm³.⁽¹⁸⁾ Although the mean value of platelets in shock cases is around 20,000/mm³, this is not always associated with severe bleeding⁽¹⁸⁾. The risk of massive bleeding appears to be high in cases with prolonged shock. Bone marrow studies (see above) show suppression of all elements, including megakaryocytes which could be an early cause of thrombocytopenia. Nevertheless, as the platelet count reaches its nadir, marrow production of platelets resumes. Therefore, the major cause of thrombocytopenia is most likely increased peripheral consumption and destruction. The destruction appears to be mediated by complement activation, as binding of platelets to C3g fragments⁽¹⁵⁾ and to viral antigens has been demonstrated⁽²⁴⁾. Impaired platelet function has been shown. Mitrakul et al observed a shortened platelet survival time and a defect in platelet ADP release in children with DHF⁽¹⁶⁾. Srichaikul et al showed that the levels of β thromboglobulin and platelet factor 4 (PF4), which are markers of platelet degranulation, are increased during the acute phase of DHF⁽²¹⁾. These findings indicate that most circulating platelets are stimulated, then become exhausted and are unable to function normally. Mitrakul et al have shown that the destroyed platelets during the acute phase of DHF were sequestered primarily in the liver, with a shift to the spleen as in normal circumstances when the patient recovered⁽¹⁶⁾.

Vasculopathy

The positive tourniquet test and skin petechiae appear early in the febrile phase before thrombocytopenia occurs. The skin biopsy specimens from areas with petechiae show lesions in the microvasculature of dermal papillae⁽⁶⁾. As mentioned above, this vascular change, which causes hemorrhage in the form of diapedesis of erythrocytes, is probably localized to capillaries and is different from those vascular changes that cause leakage⁽³⁾. In individuals with petechial rashes, skin biopsy shows the infiltration of vessel walls by lymphocytes and mononuclear phagocytes, some of which

contain dengue antigen. Deposits of IgM, complement and fibrinogen have also been observed. The lesions are similar to those seen in the antibody-dependent Arthus reactions except for the lack of necrotizing vasculitis⁽⁶⁾. The early vasculopathy may be a direct effect of the virus while the petechial rashes commonly seen in convalescence is probably mediated by an immune mechanism.

Coagulopathy

Most studies on the mechanisms of bleeding in DHF identified consumptive coagulopathy in a large proportion of cases^(8,11,16,20). Almost all severe cases with shock have coagulopathy, manifested by a prolonged partial thromboplastin time. Srichaikul et al performed the definitive study of intravascular clotting during DHF by measuring fibrinogen metabolism and found a rapid consumption of fibrinogen in 8 out of 12 cases of DHF without shock indicating that shock *per se* did not initiate coagulation. However, in cases with shock the consumption of fibrinogen was highest and the half-life of fibrinogen was shortened during the actual shock phase⁽²⁰⁾. In this study there was no evidence of abnormal fibrinolysis. In a study from Indonesia, thrombocytopenia, decreased plasma levels of fibrinogen and factor VIII, and elevated levels of fibrin degradation product D-dimer (FDP-D) were noted⁽⁸⁾. More often coagulopathy becomes apparent as fever subsides and plasma leakage becomes evident. In general, mild decreases in levels of factor II, V, VII, VIII IX, X and XII are seen whereas hypofibrinogenemia and thrombocytopenia are far more prominent^(16,20). The observed reduction in plasma coagulation factors may result from either intravascular consumption or impaired synthesis by the liver. The results of most studies of the mechanism of bleeding in DHF/DSS have implicated consumptive coagulopathy in a large proportion of cases. The finding of mild changes in liver function and normal or slightly prolonged prothrombin time in most of these cases support the role of consumptive coagulopathy. The study of fibrinogen consumption has been supported by the association between DHF and coagulopathy. In general, consumptive coagulation is not a major cause of bleeding as there is no concurrent activation of fibrinolytic system. Nonetheless, severe DIC does occur in DHF, usually with profound shock, with intractable acidosis and sometimes with markedly elevated hepatic transaminases^(10,19,22). Massive hemorrhage invariably occurs in these cases. Among fatal cases, intravascular thrombi have been detected⁽²²⁾.

In summary, hemostatic derangements in DHF appear to be multifactorial, involving vasculopathy (capillaries and venules), thrombocytopenia, platelet dysfunction and coagulopathy. These disorders may be due to direct effects of the virus on cellular functions or through activation of immune and inflammatory pathways. Rapid recovery with uneventful convalescence in cases of shock, with appropriate treatment before the stage of irreversibility has been reached, favor immune pathogenetic mechanisms. Understanding the pathogenesis of DHF is one of the most important subjects in dengue research. It will lead directly to more

effective treatment of patients with DHF, particularly those with complex hemostatic disorders. There are still a great number of research questions in the pathogenesis of DHF/DSS: It is hoped that with modern advanced technology along with cooperation from all disciplines concerned, these challenging problems can be solved.

References

1. Anon. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control. Second Edition. World Health Organization, Geneva, 1997.
2. Bhamarapravati N, Tuchinda P, Boonyapaknavik V. Pathology of Thai hemorrhagic fever: a study of 100 autopsy cases. *A Trop Med Parasitol* 1967; 61:500-510.
3. Bhamarapravati N. Hemostatic defect in dengue hemorrhagic fever. *Rev Inf Dis*, 1989; 11,S 826-824.
4. Bierman HR, Nelson ER. Hematodepressive virus diseases of Thailand. *Ann. Intern. Med.*, 1965;62:867-884.
5. Bokisch VA, Top FH Jr., Russell PK, Dixon FJ, Muller Eberhard HJ. The potential pathogenic role of complement in dengue hemorrhagic shock syndrome. *N Engl J Med*, 1973; 289: 996-1000.
6. Boonpucknavig S, Boonpucknavig V, Bhamarapravati N, Nimmannitya S. Immunofluorescence study of skin rash in patients with dengue hemorrhagic fever. *Ach Pathol Lab Med* 1979; 103: 463-466.
7. Boonpucknavig S, Lohachitranond C, and Nimmannitya S., The pattern and nature of the lymphocyte population response in dengue hemorrhagic fever. *Am J Trop Med Hyg* 1979; 28: 885-889.
8. Funahara Y, Shirahata A, Sumarmo, Setaibudy-Dharma R. DHF characterized by acute type DIC with increased vascular permeability. *Southeast Asian J Trop Med Pub Hlth* 1987; 18:346-350.
9. Halstead SB. The pathogenesis of dengue: Challenges to molecular biology. *Science* 1988; 239: 476-481.
10. Innis BL. Dengue and dengue hemorrhagic fever. In *Exotic Viral Infections*. Ed. J.S. Porterfield, Chapman and Hall Medical, New York 1995: pp 103-146.
11. Isarangura PB, Pongpanich B, Pintadit P, Phanichayakarn P, Valayasevi A. Hemostatic derangement in dengue hemorrhagic fever in children. *Southeast Asian J Trop Med Pub Hlth* 1987; 18: 331-339.
12. Kurane I, Innis BL, Nimmannitya S, et al. Human immune response to dengue viruses. *Southeast Asian J. Trop Med Pub Hlth* 1990; 21: 658-662.
13. Kurane T, Ennis FA. Immunopathogenesis of dengue virus infections. In: *Dengue and Dengue Hemorrhagic Fever*. Eds Gubler DJ, and Kuno G. CAB International, Wallingford UK, New York USA p.p. 273-290.
14. Malasit P. Complement and dengue hemorrhagic fever/dengue shock syndrome. *Southeast Asian J. Trop Med Pub Hlth* 1987; 18: 316-320.
15. Malasit P. Mongkolsapaya J, Kalayanarooj S, Nimmannitya S. Surface associated complement fragment (C3g) on platelets from patients with dengue infection. *Southeast Asian J Trop Med Pub Health (abstract)* 1990; 21: 705.
16. Mittrakul C. Bleeding problems in dengue hemorrhagic fever: Platelet and coagulation changes. *Southeast Asian J. Trop Med Pub Hlth* 1987; 18: 407-412.
17. Na Nakorn, Suingdumrong A, Pootrakul S, Bhamarapravati N, Bone marrow studies in Thai hemorrhagic fever. (Summary) *Bull WHO*. 1966; 35: 54-55.
18. Nimmannitya S. Clinical spectrum and management of dengue hemorrhagic fever. *Southeast Asian J. Trop Med Pub Hlth* 1987; 18 : 392-397.
19. Nimmannitya S, Thisayakorn U, Hemsrichart V. Dengue hemorrhagic fever with unusual manifestations. *Southeast Asian J Trop Med Pub Hlth* 1987; 18: 398-406.
20. Srichaikul T, Nimmannitya S, Arthchararit N, Siriasawakul T, Sungpeuk P. Fibrinogen metabolism and disseminated intravascular coagulation in dengue hemorrhagic fever. *Am J Trop Med Hyg* 1977; 26: 525-532.
21. Srichaikul T, Nimmannitya S, Sripaisarn T, Kamolsilpa M and Pulgate C. Platelet function during the acute phase of dengue hemorrhagic fever. *Southeast Asian J. Trop Med Pub Hlth*. 1989; 20: 19-25.
22. Srichaikul T, Punyagupta S, Nitiyanant P, and Alkarawong K. Disseminated intravascular coagulation in adults dengue hemorrhagic fever: report of three cases. *Southeast Asian J. Trop: Med Pub Hlth* 1975; 6: 106-114.
23. Suvatte V. Immunological aspects of dengue hemorrhagic fever: studies in Thailand. *Southeast Asian J. Trop Med Pub Hlth* 1987; 18: 312-315.
24. Wang S, Patarapotikul J, Innis BL, Anderson R. Antibody enhanced binding of dengue 2 virus to human platelets. *Virology* 1992; 223: 254-257.