Hemostatic derangement in Dengue infection

By
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• Overview of Severe Dengue
• Hematologic and Hemostatic Changes in Severe Dengue:
  Thrombocytopenia and platelet dysfunction
  Vasculopathy and endothelial dysfunction
  Changes in vWF parameters, ADAMTS13 and multimer
  Activation of coagulation and fibrinolysis
• Management of significant bleeding in severe dengue
### 1997 WHO dengue classification

<table>
<thead>
<tr>
<th>Classical Dengue Fever (DF)</th>
<th>Fever, headache, retro-orbital pain, myalgia, arthralgias, +/- Haemorrhagic manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHF Grade I</td>
<td>Thrombocytopenia Haemoconcentration</td>
</tr>
<tr>
<td>DHF Grade II</td>
<td>Spontaneous bleeding</td>
</tr>
<tr>
<td>DHF Grade III</td>
<td>Pulse pressure 20 mmHg, Hypotension, cold clammy skin, restless</td>
</tr>
<tr>
<td>DHF Grade IV</td>
<td>Profound shock, Undetectable BP and pulse</td>
</tr>
</tbody>
</table>
Fever of 2–7 days plus:

- Severe plasma leakage
- Significant bleeding
- Impaired consciousness
- Severe organ impairment (AST or ALT > 1,000)
- Severe involvement of the heart or others organs

Severe Dengue: guidelines for diagnosis, treatment  WHO 2009
Pathophysiology in severe dengue

Risk factors for severe dengue:
- Young age
- Female
- High body mass index
- Virus strain
- Genetic variants
- Secondary infection by different serotypes

Viremia

Transient and reversible imbalance of inflammatory mediators, cytokines, and chemokines
Endothelial cell dysfunction

Derangement of coagulation system

Plasma leakage
Shock
Bleeding
Hematologic and Hemostatic Changes in Severe Dengue
Peripheral blood changes in DHF

Correlation between WBC and PMN

Correlation between WBC and ATL

Peripheral blood changes in DHF

Febrile  Toxic  Convalescent

Days of illness

WBC count

PMN count

WBC count

Atypical lymphocyte count

Days of illness

WBC count

Atypical lymphocyte count
Correlation between Hematocrit and platelets in DHF

Phases of the disease
- Febrile
- Toxic
- Convalescent

Day of the illness

Hematocrit (%)

Platelet count

ISTH WORKSHOP on Thrombosis and Haemostasis November 4-7, 2017 BANGKOK
Thrombocytopenia

I. Peripheral destruction or increased utilization

- Dengue 2 virus can bind to human platelet, and result in immune-mediated platelet destruction
- Consumption of platelet during the process of consumptive coagulopathy

II. Decreased production; dengue-virus-induced

BM suppression depressed platelet synthesis

La Russa VF. Baillieres Clin Haematol 1995; 8(1): 249-70
Platelet dysfunction

- **Decrease Platelet aggregation** after stimulation with 5 µm M ADP in DHF patients during febrile or early convalescent period
- 9 in 10 children (90%) with DF and all of children with DHF have **decrease platelet aggregation with ADP** (DF 60%, DHF 100%), **ristocetin** (DF 40%, DHF 100%), **collagen** (DF 70%, DHF 100%) and **arachidonic acid** (DF 90%, DHF 66.7%)
- **Plasma levels of platelet factor 4 and beta thromboglobulin** were increased during the acute phase of DHF

Tanyong B, Sosothikul D. Abnormal Platelet Aggregation of Dengue fever in Thai Children 2011
In vitro study of dengue infection showed that platelet-derived IL-1B was chiefly released in microparticles and correlated with sign of increased vascular permeability.

Platelet derived microparticles in Dengue infection

(Dengue : N=20)

P = 0.007

(N=40)

Sosothikul D, et al Poster presentation at European Hematology Association 2014, Milan, Italy
Interleukin-1 beta in Dengue infection

P < 0.001

Sosothikul D, et al Poster presentation at European Hematology Association 2014, Milan, Italy
Platelet Activation in Dengue Infection

- The levels of PDMP, and IL-1β were significantly increased in patients with dengue infection compared to the unaffected controls.

- Platelet activation can be one of the mechanisms that lead to platelet dysfunction and increased vascular permeability in patients with dengue infection.

Vasculopathy

- Plasma leakage, due to an increase in capillary permeability, is a cardinal feature of DHF but is absent in dengue fever.
- Appears to be due to endothelial cell dysfunction rather than injury, as electron microscopy demonstrated a widening of the endothelial tight junctions.
- Dengue virus-infected monocytic cells produce TNF-alpha and activate endothelial cells in vitro.

Activation of endothelial cells in dengue infection

Soluble thrombomodulin

Von Willebrand Factor antigen

Changes in ADAMTS 13 and VWF parameters in dengue infection

VWF Multimers in DHF

Coagulopathy in DHF

Dengue/Cytokines

- Activated endothelial cells
- Platelets sequestration

Activated endothelial cells

- TM
- t-PA
- vWF

TF/FVIIa

Prothrombin

Thrombin

Thrombin anti-thrombin complex (TAT)

AT: anti-thrombin III

FXIa

FIXa+FVIII

FXa+FV

- TAFIa

- TM

Fibrinogen

D-Dimer

- Plasmin

- Plasminogen

- PAI-1

Fibrin

Fibrinolysis

TM: thrombomodulin
vWF: von Wellibrand factor
t-PA: tissue plasminogen activator inhibitor
PAI: plasminogen activator inhibitor
TF: tissue factor
TAT: thrombin anti-thrombin complex
AT: anti-thrombin III
TAFI: thrombin activatable fibrinolysis inhibitor

CT = clotting time, CFT = clot formation time, MCF = maximum clot firmness
### Coagulation tests in dengue patients during febrile phase

<table>
<thead>
<tr>
<th>Tests</th>
<th>Controls (n = 30)</th>
<th>Dengue fever (n = 22)</th>
<th>Dengue hemorrhagic fever (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematocrit (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>41.1 ± 3.3</td>
<td>37 ± 4.4**</td>
<td>44.1 ± 3.4</td>
</tr>
<tr>
<td>Range</td>
<td>(34 - 46.4)</td>
<td>(28.2 - 45.8)</td>
<td>(37.3 - 50.9)</td>
</tr>
<tr>
<td><strong>Platelets (x10⁹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>289.4 ± 832</td>
<td>99.6 ± 48.3*</td>
<td>40.8 ± 31.4</td>
</tr>
<tr>
<td>Range</td>
<td>(152 - 485)</td>
<td>(0 - 196.2)</td>
<td>(0 - 103.6)</td>
</tr>
<tr>
<td><strong>Prothrombin time (sec)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>12.5 ± 1.3</td>
<td>14 ± 1.8*</td>
<td>16.3 ± 4.6</td>
</tr>
<tr>
<td>Range</td>
<td>(10.6 - 14.7)</td>
<td>(10.4 - 17.6)</td>
<td>(7.1 - 25.5)</td>
</tr>
<tr>
<td><strong>APTT (sec)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>35.9 ± 3.1</td>
<td>42 ± 6.5*</td>
<td>48.4 ± 9.6</td>
</tr>
<tr>
<td>Range</td>
<td>(29.7 - 40.3)</td>
<td>(29 - 55)</td>
<td>(29.2 - 67.6)</td>
</tr>
<tr>
<td><strong>Fibrinogen (mg/ dL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>429.4 ± 110.3</td>
<td>307 ± 70.9</td>
<td>306.3 ± 94</td>
</tr>
<tr>
<td>Range</td>
<td>(220 - 678)</td>
<td>(165.2 - 448.8)</td>
<td>(118.3 - 494.3)</td>
</tr>
</tbody>
</table>

* p <0.05 and ** p < 0.001 : Dengue fever (DF) vs Dengue hemorrhagic fever (DHF)
Results from thromboelastometry

CT = clotting time, CFT = clot formation time, MCF = maximum clot firmness

Sosothikul D, et al. Oral Poster presentation at ISTH meeting 2013
Results from thromboelastometry

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Sosothikul D, et al. Oral Poster presentation at ISTH meeting 2013
Thromboelastometry in Dengue

- ROTEM® showed significant changes in hemostasis in both groups of dengue patients, and it was correlated well with standard coagulation studies and severity of the disease.
- ROTEM® can early detect abnormal fibrinogen function in DHF patients.
- It may become a useful bedside tool for an early detection and a quick guide to choose appropriate blood products in treatment of DHF patients with bleeding.

Sosothikul D, et al. Oral Poster presentation at ISTH meeting 2013
Management of significant bleeding in DHF
Risk factors for Hemorrhage in DHF

- A study of risk factors for hemorrhage in 114 patients with DHF/DSS showed no correlation between bleeding and platelet count.
- The strongest risk factor for hemorrhage were prolonged duration of shock and a low level of hematocrit at the time of shock.

Management of significant bleeding

- Significant internal bleeding should be suspected in patients with signs of intravascular hypovolemic without elevation of hematocrit
  \[ \text{PRC} \, 5 \, \text{ml/kg} \] and clinical response/post transfusion hematocrit should be monitored

- Do not wait for the hematocrit to drop too low before deciding on blood transfusion

Thomas L, et al Transfusion 2009; 49:1400
Comprehensive guidelines for prevention/control DHF WHO Regional Office for Southeast Asia 2011
Transfusion in dengue with hemorrhage

**FFP and/or platelet - no different in bleeding**
Increase pulmonary edema, longer hospitalization

**Platelet**
- Benefit in DIC with platelet < 50,000 or undergoing procedure
- Platelet 0.1 unit/kg/dose
- Not indicate for prevent spontaneous bleeding

**FFP**
- PT or aPTT ratio > 1.5 + DIC or severe bleeding
- FFP 10-15 ml/kg

Update on pediatric infectious diseases 2016
NG tube/ foley catheter:
- Great care should be taken when inserting a NG tube or bladder catheters
- A lubricated orogastric tube may minimize the trauma during insertion

Central line
- Should be done with USG guidance or by an experienced person
• rFVIIa may have role in case of massive bleeding unresponsive to conventional blood component therapy.
• rFVIIa enhances thrombin generation and also enhances the activity and function of both patients and transfused platelets.

Dengue and prophylactic transfusions

- 106 DSS children with thrombocytopenia and coagulopathy, there was no difference in hemorrhage between patients who received preventive transfusions compared with those who did not.
- Patients who received transfusion had higher frequency of development of pulmonary edema and increased length of hospitalization.
- Preventive transfusions did not produce sustained improvements in the coagulation status in DSS.

Conclusions

• Bleeding in DHF patient is caused by vasculopathy, thrombocytopenia, platelet dysfunction, abnormal VWF multimers and coagulopathy (DIC)
• ROTEM® may become a useful bedside tool for an early detection and a quick guide to choose appropriate blood products in treatment of DHF patients with bleeding
• Prophylactic platelet transfusions have not been shown to be effective at preventing or controlling hemorrhage
Conclusions

- Platelet transfusion should be reserved for dengue patients with major bleeding
- Early recognition of severe dengue, with prompt correction of hemodynamic status, remains the mainstay for good clinical outcome
THANK YOU FOR YOUR ATTENTION