



Biomarkers of severe dengue disease – a review

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Abstract

Dengue virus infection presents a wide spectrum of manifestations including asymptomatic condition, dengue fever (DF), or severe forms, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) in affected individuals. The early prediction of severe dengue in patients without any warning signs who may later develop severe DHF is very important to choose appropriate intensive supportive therapy since available vaccines for immunization are yet to be approved. Severe dengue responses include T and B cell activation and apoptosis, cytokine storm, hematologic disorders and complement activation. Cytokines, complement and other unidentified factors may transiently act on the endothelium and alter normal fluid barrier function of the endothelial cells and cause plasma leakage. In this review, the host factors such as activated immune and endothelial cells and their products which can be utilized as biomarkers for severe dengue disease are discussed.

Keywords: Severe dengue, Biomarkers, Immune activation, Endothelial activation

Introduction

Dengue is a mosquito borne viral infection found in tropical and sub-tropical regions of the world and is caused by one of the four serotypes of dengue viruses (DENV1-DENV4). An increase in infection has been seen in recent years due to many factors including urbanization and air travel. Over 2.5 billion people of the world's population are now at risk for dengue. The consequences of DENV infection range from asymptomatic condition, dengue fever (DF), or severe forms, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Severe dengue is characterized either by plasma leakage, fluid accumulation, respiratory distress, severe bleeding, or organ impairment [1]. Clinical manifestations offer the earliest markers in predicting severe dengue disease. A recent meta-analysis of signs and symptoms of severe dengue shows that bleeding, nausea and vomiting, abdominal pain, skin rashes, and hepatosplenomegaly are associated with severe dengue disease [2]. Patients with dengue fever are clustered into two groups: one with warning signs including abdominal pain, mucosal bleeding and liver enlargement that

warrant ICU admission and the other without those signs [1, 2]. Early prediction of severe dengue in patients without any warning signs who may later develop severe DHF is very important to give the best supportive care since approved vaccines for immunization are yet to be commercialized. An ideal biomarker should be able to identify individuals who are at risk of developing severe dengue.

The mechanism by which only a few DENV infected individuals progress to severe dengue disease is poorly understood. The host immune responses have been considered as the major factor responsible for dengue pathogenesis. The process of plasma leakage, shock and hemorrhagic manifestations initiated by enhancing infection with DENV virus with the help of opsonizing antibodies, resulting in an altered immune response which trigger T cell activation and release of cytokines and chemical mediators has been a risk factor in secondary infection [3, 4]. However, undefined factors could play a role in the development of severe dengue in individuals with naïve primary infection and immune non-responders [5]. Dengue patients show fever symptoms during peak of viremia while DHF/DSS appears during the time when the virus has been cleared from the circulation suggesting severe dengue disease is most likely associated with immunopathology. Thus, the host immune response components including cells, cytokines,

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complements and other cellular mediators can serve as biomarkers of severe disease [6, 7]. It is reported that the macro-morphology of endothelial lining remains intact while the functionality of the endothelial cells is altered by activation which leads to vascular permeability resulting in plasma leakage [8]. Therefore, endothelial activation markers such as expression of adhesion molecules and receptors can also serve as biomarkers of severe dengue disease [9, 10]. In this review, the various host immune and endothelial activation markers and biochemical and genetic markers are reviewed for their utility as potential biomarker of severe dengue disease.

Immune activation markers as predictors of severe dengue disease

Number and activation status of immune cells

DENV has been shown to infect a wide range of cells including dendritic cells (DCs), monocytes, lymphocytes, hepatocytes, endothelial cells (ECs) and mast cells *in vitro* [6]. Although the role of these cells in DENV infection remains less clear *in vivo*, activation of memory T cells resulting in cascades of inflammatory cytokines and other chemical mediators that trigger death of target cells through apoptosis is a critical element contributing to severe dengue [11]. DCs and macrophages are the primary targets of DENV infection [12, 13]. Both the absolute number and frequency of circulating myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) were decreased early in acute viral illness in children but not in adults who subsequently developed DHF and decreased level of pDCs was associated with higher viremia levels [14, 15]. Activated DCs may contribute to vascular leak through the production of TNF- α , IFN- γ and matrix metalloproteinases-2, 3 and 9 [16, 17]. Studies show that CD4 T cell, CD8 T cell, NK cell and $\gamma\delta$ T cell counts were significantly decreased in DHF compared to DF early in the course of illness [18]. The CD8 T cells and NK cells from dengue patients displayed activation markers such as CD69, HLA-DR, CD38 and cytotoxic granule TIA-1 and cell adhesion molecules CD44 and CD11a during the acute phase [19, 20]. The decreased numbers of lymphocytes could be due to increased apoptosis of peripheral blood mononuclear cells observed during DENV infection evidenced by the presence of increased plasma levels of soluble CD95, a mediator of apoptosis, and down-regulation of the antiapoptotic protein Bcl-2 in these cells [21, 22]. Cross-reactive memory T cells that are highly activated with increased levels of cytokine producing capacity was observed in patients with acute dengue disease. Activation of cross-reactive low affinity T-cells results in copious amounts of cytokine and chemokine production such as IFN- γ , TNF- α , IL-1, IL-6, IL-8, IL-10, CCL2 (MCP-1) and CCL5 (RANTES) [23, 24].

Activation of mast cells and increased levels of urinary histamine, which is a major product of mast cells were observed in dengue patients and the levels correlated with disease severity [25]. Several data indicated that virus stimulated mast cells selectively produced and secreted a variety of mediators including chemokines, cytokines, lipid mediators, and granule associated products. Elevated levels of secreted CCL3 (MIP-1 α), CCL4 (MIP-1 β) and CCL5 were observed following infection of human mast cell lines [26, 27]. Substantial levels of tryptase and chymase were found in mast cells and these proteases are considered to be selective markers of mast cell activation. Plasma levels of both tryptase and chymase were increased significantly in DHF/DSS compared with DF. [28] Thrombocytopenia is one of the clinical hallmarks for dengue patients. There are many mechanisms leading to the depletion of platelets in affected subjects, these include direct infection of megakaryocytes by DENV as well as platelet destruction due to nonstructural protein 1 (NS1) binding and platelet-associated antibodies [29–31]. Thrombocytopenia is best used as a marker of severe disease particularly when it is <100,000 cells/c.mm and serve as an indicator of prognosis during the course of the disease [18, 32, 33]. Since thrombocytopenia is seen in both DF and DHF patients, a platelet count of 60,000 cells/c.mm serves as a better cut-off in identifying more severe cases [7].

Increased levels of cytokines and chemokines

Patients with DHF/DSS present a 'cytokine storm,' with high levels of circulating cytokines and chemokines. Therefore, serum cytokine and chemokine levels can serve as a laboratory tool for predicting severe disease. T cells, NK cells, monocytes, macrophages, hepatocytes and ECs have been shown to contribute to the increased production of cytokines and chemokines. Increased levels of IFN- γ , TNF- α , IL-1 β , IL-4, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IL-17, IL-18, macrophage migration inhibitory factor (MIF) and chemokines CCL2, CCL4, CCL5, and CXCL10 (IP-10) have been reported in patients with DHF when compared to DF. [34–38] Studies show that elevated levels of IL-6, IL-10, IFN- γ , MIF, and CCL-4 could be used as potential predictors of severe dengue [39–46].

Complement, antibodies and other soluble factors

Complement activation and an increase in complement protein products correlate with severe dengue disease [47]. Large amounts of C3, C3a and C5a have been detected in DENV-infected patients and determining their levels in serum is important since these anaphylatoxins direct the lysis of infected cells and mast cell degranulation leading to histamine release [48, 49]. An increase in the number of B lymphocytes was

demonstrated in DHF. [18] Total and dengue-specific IgE antibody levels were higher in patients with DHF and DSS compared with those with DF. [50] NS1 is an immunogen and high concentrations of anti-NS1 antibodies have been found in severe disease. Antibodies to NS1 can cross-react with human ECs and platelets and cause vascular permeability with production of nitric oxide (NO) and apoptosis [51, 52]. Determining the levels of dengue-specific IgE, anti-platelet and anti-EC antibodies might be used as biomarkers of severe dengue disease. Soluble factors are more stable and have the potential to serve as biomarkers. Increased levels of soluble receptors such as sTNFR1, sCD4, sCD8, sIL-2R were reported in DHF patients when compared to those with DF and that their levels correlated with disease severity [18, 53]. Release of sTNFR may be an early and specific marker of the endothelial changes that cause DSS [42]. The IL-1 receptor-like-1 protein (IL1RL1), also known as ST2, is a member of the IL1R/Toll-like receptor (TLR) superfamily. Increased serum sST2 was found in patients having secondary infection and DHF patients compared to DF patients and may be a predictive marker of dengue severity [44, 54].

Endothelial activation markers as predictors of severe dengue disease

Endothelium is the ultimate target of permeabilizing responses and DENV influence on ECs may be direct or indirect by the release of mediators from infected or activated immune cells. DENV antigens associated with ECs were observed in autopsy samples in liver, spleen, kidney and lungs from DHF/DSS patients and infection of primary ECs by DENV has been reported [55, 56]. DENV infected ECs produce chemokine and cytokine responses that activate or recruit immune cells to the endothelium [57, 58]. Uncontrolled and persisted activation of endothelium leads to vascular permeability, microvascular thrombosis and inflammation and thus the components of activated endothelium in serum and/or plasma can serve as biomarkers of severe dengue disease [10, 59]. Under normal circumstances, the serum concentration of angiopoietin-1 (Ang-1) exceeds Ang-2, which are the mediators of endothelial function. Indonesian children showed decreased Ang-1 and increased Ang-2 levels in DHF/DSS [60]. Von Willebrand factor (vWF), a disintegrin and metalloproteinase with thrombospondin-1-like domains (ADAMTS-13) and thrombomodulin (TM) are components of coagulation system. Both vWF and ADAMTS-13 are synthesized and released by the ECs and the former stabilizes the adhesion of platelets at the site of vascular injury while the latter inhibits thrombin formation [61, 62]. Children with acute dengue infection have elevated vWF levels and the levels were particularly higher in DSS patients

while ADAMTS-13 levels were decreased in children with DHF/DSS [63]. TM is present in large quantities on the surface of endothelium and acts as an anticoagulant. Serum level of sTM is proposed as diagnostic and prognostic marker of endothelial activation and dysfunction. Children from Thai and Vietnamese population show increased sTM levels particularly in children with severe dengue disease [64, 65]. Soluble forms of cell surface molecules such as sE-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are released from ECs after activation and are used as diagnostic and prognostic markers in infectious diseases. sICAM and sVCAM-1 but not sE-selectin were found to be elevated in DSS as compared to DF and their levels do not differ between DHF and DSS [20, 66, 67]. The vascular endothelial growth factor (VEGF) is a potent stimulator of endothelial permeability and promotes proliferation, migration and survival of ECs. VEGF may contribute to inflammation and coagulation by inducing the expression of cell adhesion molecules that promotes adhesion of leukocytes. VEGFR1 and VEGFR2 are expressed on the ECs [68]. The VEGF and VEGFR1 plasma levels were significantly higher in DHF and DSS than those in DF while VEGFR2 levels were decreased in DHF/DSS compared to DF. [28, 69] Thus, increase in Ang-2, vWF, sVCAM, VEGF and VEGFR1 levels and decrease in Ang-1, ADAMTS-13 and VEGFR2 levels might serve as biomarkers of severe dengue disease.

Biochemical markers as predictors of severe dengue disease

Several biochemical compounds are shown to be either elevated or decreased in serum/plasma of patients with severe dengue and quantifying them might serve as biomarkers of severe dengue disease. Levels of total plasma cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were significantly decreased in children with the severest disease compared with patients with mild DHF. [70] Microbial translocation occurs during severe DENV infection and lipopolysaccharide (LPS) levels are significantly increased in dengue patients which is indicated by elevated levels of LPS binding protein (LBP) and soluble CD14 (sCD14). Elevated LPS levels in dengue patients were found to correlate with clinical disease severity [38]. Liver injury is associated with severe dengue disease with the increase in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase, alkaline phosphatase, and serum albumin concentrations. Reports showed that the AST and ALT levels were high in severe disease and may serve as predictors of severe disease [32, 33, 71]. Children with DSS and liver injury have lower zinc levels and the low levels were probably

caused by loss from diarrhea and from zinc translocating to liver cells [72]. The Inter- α Inhibitor Proteins (IaIp) belong to a family of serine protease inhibitors and its concentrations in pediatric patients suffering from severe DENV infection were significantly lower than in patients with mild DF and healthy controls [73]. NO is known to have a strong immunoregulatory role and in adjusting the diameter of blood vessels, remodeling blood vessels, inhibiting leukocyte adhesion, platelet aggregation, and contractile cell proliferation [74]. Serum NO levels in DHF patients were shown to be significantly lower than those of the DF patients [33, 75]. Thus, increased levels of LPS, AST, ALT and decreased levels of lipids, IaIp and NO might serve as markers of severe dengue disease.

Host genetic markers as predictors of severe dengue disease

DNA microarrays have been used as a tool to identify genes and predict patient's outcome for bacterial and viral infection [76]. Differential expression of genes has been shown in DF and DHF/DSS patients and hence can serve as markers of severe dengue [77, 78]. A recent

study showed that analysis of seven genes (LOC286087, SLC4A4, PSPH, MYOM2, CACNA2D3, CD244 molecule, SMAD5) could possibly predict severe dengue since their expression profile was significantly lower in DHF patients compared to patients with DF. [79] DENV infection can trigger apoptosis in a variety of cell types and apoptotic cells are the main source of cell-free DNA into the circulatory system [21, 80]. Circulating DNA levels were significantly higher in patients with DENV infection than with other febrile illnesses and the increase of DNA levels can be correlated with disease severity [81].

Conclusion

The usage of the four classes of biomarkers has advantages and limitations. Molecular markers can be accurate. However, it involves high cost for the sequencer and reagents. Immunological markers which are also seen in other inflammatory diseases require flow cytometry analysis. Although the cost of the instrument has come down, the costs of reagents are still very high for dengue endemic countries. Endothelial activation and biochemical markers are easy to estimate and the cost is low. However, the levels are modified in other disease

Table 1 Biomarkers of severe dengue disease

Class	Biomarker		Change	Reference
1. Immune activation markers	Cells	Plasmacytoid dendritic cells	Decrease	14
		Lymphocytes	Decrease	18, 20
		Platelets	Decrease	18, 32, 33
	Cytokines	IL-10	Increase	32, 40, 44
		MIF	Increase	39, 45
	Chemokines	CXCL-10	Increase	46
	Complements	C3a, C5a	Increase	48, 49
	Soluble receptors	sCD4/8, sIL-2R, sTNFRII	Increase	53
	Proteases	Tryptase and chymase	Increase	28
	2. Endothelial activation markers	Mediators of endothelial function	Angiopoietin-1	Decrease
Angiopoietin-2			Increase	60
Coagulation pathway components		von Willebrand factor	Increase	63
		ADAMTS-13	Decrease	63
		sThrombomodulin	Increase	64, 65
Cell surface adhesion molecules		sICAM, sVCAM	Increase	20, 66, 67
Permeability mediators		VEGF, VEGFR1	Increase	28, 69
	VEGFR2	Decrease	28, 69	
3. Biochemical markers	Lipids	Total cholesterol, HDL, LDL	Decrease	70
	LPS	LPB, CD14	Increase	38
	Liver enzymes	AST, ALT	Increase	32, 33, 71
	Serine protease	IaIp	Decrease	73
	Other soluble substances	Nitric oxide	Decrease	33, 75
4. Genetic markers	Gene profile	Certain gene expression	Decrease	77–79
	Circulating cell free-DNA		Increase	81

conditions as well. Therefore, determination of combination of biomarkers will be beneficial in predicting severe dengue disease. As shown in the table 1, information on lymphocyte and platelet counts, levels of IL-10 and MIF, levels of AST and ALT, levels of gene expression and cell free DNA will aid in predicting severe dengue. With the advances in techniques and equipments, multiple biomarker analysis is possible with small amounts of patient serum samples. However, kinetics and cut-off levels need to be established particularly for children and patients in dengue endemic regions.

Competing interest

The authors declare that they have no competing interests.

Authors' contribution

DVJ drafted the manuscript. YSL and GCP made critical revisions and gave suggestions during the preparation. All authors read and approved the final manuscript.

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