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Serum angiopoietin-2 and soluble VEGF receptor 2 are surrogate markers for plasma leakage in patients with acute dengue virus infection

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ABSTRACT

Background: Endothelial cell dysfunction is believed to play an important role in the pathogenesis of plasma leakage in patients with acute dengue virus (DENV) infection. Several factors, produced by activated endothelial cells, have been associated with plasma leakage or severe disease in patients with infectious diseases.
Objectives: The aim of this study was to investigate which of these markers could serve as a surrogate marker for the occurrence of plasma leakage in patients with acute DENV infection.
Study design: A case-control study was performed in patients with acute DENV infection in Santos, Brazil. Plasma leakage was detected with X-ray and/or ultrasound examination at admission. Serum levels of soluble endoglin, endothelin-1, angiopoietin-2, VEGF, soluble VEGFR-2, MMP-2, MMP-9, TIMP-1 and TIMP-2 were determined using commercially available ELISAs.
Results: Increased levels of angiopoietin-2, endothelin-1 and MMP-2 and decreased levels of soluble VEGFR-2 were significantly associated with the occurrence of plasma leakage. An unsupervised cluster analysis confirmed that angiopoietin-2 and soluble VEGFR-2 were strongly associated with clinical apparent vascular leakage.
Conclusion: Angiopoietin-2 and soluble VEGFR-2 can serve as surrogate markers for the occurrence of plasma leakage in patients with acute DENV infection.

1. Background

Dengue virus (DENV) is a flavivirus, which is transmitted by the bite of a mosquito. A recent study showed that 390 million persons are infected with DENV each year, of which 96 million develop clinical symptoms [1]. A hallmark of dengue disease is an increase in vascular permeability, presented as pleural effusion and/or ascitis. In severe cases, extensive plasma leakage may lead to the development of hypotension and shock [2].

Endothelial cells play a crucial role in the development of plasma leakage during DENV infection. DENV can infect endothelial cells

Abbreviations: DENV, dengue virus; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix metalloproteinases; Ang, angiopoietin; Eng, endoglin; ET-1, endothelin-1; RT-PCR, real time PCR; Chi, Chi-squared test; MWU, Mann–Whitney *U* test; F, Fisher's exact test; MV, missing value; WS−, non-severe dengue without warning signs; WS+, non-severe dengue with warning signs; HC, healthy control.

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Table 1
Characteristics of patients with and without plasma leakage.

	No plasma leakage ($N=56$)	Plasma leakage ($N=49$)	Healthy controls ($N=15$)	Significance
Sex	59% male ($N=33$)	63% male ($N=31$)	60% male ($N=9$)	$p=0.9$ (Chi)
Age*	21 (11–45)	12 (8–29)	25 (24–28)	$p=0.001$ (KW)
Day of fever*	5 (4–6)	5 (4–7) MV = 1	NA	$p=0.9$ (MWU)
2009 WHO dengue case classification	64% ($N=36$) WS– 34% ($N=19$) WS+ 2% ($N=1$) Severe	100% ($N=49$) WS+	NA	$p<0.0001$ (Chi)
Admission	52% ($N=29$) MV=2	96% ($N=47$) MV=1	NA	$p<0.0001$ (F)
Type plasma leakage	–	Ascites: 29% ($N=14$) Pleural: 16% ($N=8$) Pleural and pericardium: 55% ($N=27$)	NA	
Haemorrhagic manifestations	30% ($N=17$)	47% ($N=23$)	NA	$p=0.1$ (F)
Type haemorrhagic manifestation	70% ($N=39$) No 25% ($N=14$) Minor mucosal 5% ($N=3$) Petechiae	53% ($N=26$) No 31% ($N=15$) Minor mucosal 16% ($N=8$) Petechiae	NA	
Platelet count*	122.500 (57.250–166.500) MV = 2	42.000 (33.000–73.000)	NA	$p<0.0001$ (MWU)
Viremic	73% ($N=41$)	59% ($N=29$)	NA	$p=0.2$ (F)
Viral copy number in viremic patients (copies/ml)*	198 (122–950)	126 (100–256)	NA	$p=0.01$ (MWU)
IgG avidity*	21% ($N=12$) Not detectable 2% ($N=1$) Primary 77% ($N=43$) Secondary	2% ($N=1$) Not detectable 98% ($N=48$) Secondary	NA	$p=0.006$ (Chi)

Abbreviations: Statistical test used is the: Chi = Chi-squared test; KW = Kruskal Wallis test; MWU = Mann–Whitney *U* test; F = Fisher's exact test. MV = missing value. WS– = non-severe dengue without warning signs. WS+ = non-severe dengue with warning signs. NA = not applicable.

* Values are in median (interquartile range).

in vitro, but whether this also occurs in vivo, is still a matter of debate [3,4]. Moreover, it is not clear whether DENV causes vascular permeability by direct infection of endothelial cells or through the release of vasoactive agents by infected monocytes and macrophages, which are the primary target cells of DENV infection [4]. In vitro, direct infection of endothelial cells did not lead to an increase in permeability, while co-incubation of endothelial cells with mononuclear cells or the supernatant from DENV-infected monocytic cells did result in an increase [5,6]. This suggests that mechanisms other than direct infection may activate endothelial cells, resulting in an increase in vascular permeability. It is believed that uncontrolled endothelial activation and subsequent dysfunction contributes to the severity of dengue (reviewed in [7]).

Vascular endothelial growth factor (VEGF), initially identified as vascular permeability factor, promotes the growth, proliferation and migration of endothelial cells. VEGF is increased in DENV infected patients with plasma leakage, especially around the time of defervescence [8,9]. VEGF can be bound to sVEGFR-1 and sVEGFR-2, which are expressed predominantly on endothelial cells [10]. Levels of sVEGFR-1 were increased in patients with severe dengue, contrasting with decreased levels of sVEGFR-2 [8].

Matrix metalloproteinases (MMPs) are proteolytic enzymes that can cleave proteins of the extracellular matrix [11]. The activity of these enzymes is regulated by tissue inhibitors of matrix metalloproteinases (TIMPs). Endothelial cells produce MMP-2 and MMP-9 and also TIMP-1 and TIMP-2 [12]. Increased levels of MMP-9 were detected in patients with severe DF compared to mild DF [13]. In the same study, no significant differences were detected in MMP-2 levels between dengue fever patients and healthy controls.

Angiopoietin-1 (Ang-1) is produced by perivascular cells and has a stabilizing effect on the vascular barrier [14]. Angiopoietin-2 (Ang-2) is synthesized by endothelial cells and is a potent inducer of vascular permeability by counteracting the barrier stabilizing effects of Ang-1 [15]. Decreased levels of Ang-1 and increased levels of Ang-2 were correlated with the occurrence of plasma leakage in DENV infected patients, suggesting that an imbalance between these two proteins may be involved in endothelial dysfunction [16].

Activated endothelial cells produce a number of other proteins, including soluble endoglin (sEng) and endothelin-1 (ET-1). Upon inflammation, Eng is cleaved by MMPs and released in the circulation as sEng. sEng binds to TGF-β1 and abrogates its anti-inflammatory effects. Levels of sEng were increased in children with severe malaria [17]. ET-1 is produced by endothelial cells and is a potent vasoconstrictor and has inotropic, chemotactic and mitogenic properties [18]. Increased levels have been detected in patients with sepsis and malaria [19,20].

2. Objectives

The aim of this study was to investigate which of the following markers sEng, ET-1, MMP-2, MMP-9, TIMP-1, TIMP-2, Ang-2, VEGF and sVEGFR-2, all produced by activated endothelial cells, could serve as a surrogate marker for the increase in vascular permeability during DENV infection.

3. Study design

3.1. Clinical cohort

This cohort has been previously described [21–24]. Briefly, during the 2010 outbreak, samples were collected from patients with clinical suspected dengue presenting at the Ana Costa

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