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Host biomarkers are associated with progression to dengue haemorrhagic fever: a nested case-control study

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ABSTRACT

Objectives: Dengue represents the most important arboviral infection worldwide. Onset of circulatory collapse can be unpredictable. Biomarkers that can identify individuals at risk of plasma leakage may facilitate better triage and clinical management.
Design: Using a nested case-control design, we randomly selected subjects from a prospective cohort study of dengue in Colombia (n = 1582). Using serum collected within 96 hours of fever onset, we tested 19 biomarkers by ELISA in cases (developed dengue hemorrhagic fever or dengue shock syndrome (DHF/DSS); n = 46), and controls (uncomplicated dengue fever (DF); n = 65) and healthy controls (HC); n = 15.
Results: Ang-1 levels were lower and angptl3, sKDR, sEng, sICAM-1, CRP, CXCL10/IP-10, IL-18 binding protein, CHI3L1, C5a and Factor D levels were increased in dengue compared to HC. sICAM-1, sEng and CXCL10/IP-10 were further elevated in subjects who subsequently developed DHF/DSS ($p = 0.008$, $p = 0.028$ and $p = 0.025$, respectively). In a logistic regression model, age (odds ratio (OR) (95% CI): 0.95 (0.92-0.98), $p = 0.001$), hyperesthesia/hyperalgesia (OR; 3.8 (1.4-10.4), $p = 0.008$) and elevated sICAM-1 (>298ng/mL: OR; 6.3 (1.5-25.7), $p = 0.011$) at presentation were independently associated with progression to DHF/DSS.
Conclusions: These results suggest that inflammation and endothelial activation are important pathways in the pathogenesis of dengue and sICAM-1 levels may identify individuals at risk of plasma leakage.

1. Background

Dengue is an emerging infectious disease of global importance with 2.5 to almost 4 billion people residing in dengue endemic countries in which an estimated 50-100 million infections occur annually.[1,2] Historically, the highest risk areas for dengue fever were in Southeast Asia and the western Pacific (where approximately 75% of the global disease burden is located). However, the risk of dengue, and severe dengue, is increasing in the Americas following disease re-emergence after lapsed vector control programs.[3] Between 2000 and 2006, the majority of cases reported (68%) were registered in this region.[4] Cyclical outbreaks of dengue in the Caribbean, and Central and South America have resulted in considerable morbidity and mortality. In 2013, 2.35 million cases of dengue were reported in the Americas, of which 37,687 cases were classified as severe dengue, with Brazil and Colombia recording the highest incidence.

Dengue is an important consideration in the differential diagnosis of fever in endemic countries and in travelers returning from these regions.[5] Dengue infection presents with a wide spectrum of signs and symptoms, including rapid onset of fever that may be accompanied by myalgia, arthralgia, retro-orbital pain, facial flushing, erythema, nausea, vomiting, cutaneous

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hyperesthesia or hyperalgesia, and minor bleeding abnormalities as evidenced by a positive tourniquet test. A small percentage of individuals with dengue will develop life-threatening complications, which can include plasma leak and circulatory collapse. The clinical evolution of disease can be unpredictable making the initial clinical assessment a critical step in patient management.[6]

Dengue is a single-stranded RNA virus with four distinct serotypes (DEN-1 to -4). Risk of haemorrhage and plasma leak is generally higher in secondary infections, but other factors including age, sex, and other host and viral determinants also contribute to disease susceptibility. The prevailing hypothesis is that cross-reactive, non-neutralizing antibodies in secondary heterotypic infections facilitate viral entry into target cells, thereby increasing viral load, enhancing deleterious inflammatory responses and contributing to endothelial activation and dysfunction (reviewed in[7]). The endothelium is central to dengue pathogenesis as increased vascular permeability and fragility are defining features in severe disease. However, inflammation, coagulation and complement activation are also involved in disease pathogenesis, and alterations in soluble mediators from these pathways have been described in severe dengue infections.[8–18]

Clinical management of dengue depends on appropriate triage, referral and treatment of cases, especially in outbreaks. Although there are no specific anti-infective or immunomodulatory therapies to prevent plasma leak, appropriate fluid management and supportive care can reduce the mortality rate from over 20% to less than 1%.[3,19] With rates of severe disease on the rise, especially in the Americas,[20–22] new tools are urgently needed to improve patient triage and risk prediction of severe disease.[23] We investigated whether host biomarkers, chosen from pathways implicated in dengue pathogenesis, were altered in dengue relative to healthy controls, and whether the biomarkers assessed during the acute phase of disease were associated with the subsequent development of dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS).

2. Methods

2.1. Study Population

All four dengue virus serotypes circulate in Colombia, which reports the highest number of severe dengue cases and deaths in the region.[3,24] Bucaramanga is a metropolitan area in northeastern Colombia where the incidence of dengue ranges from 113-269 cases per 100,000 people.[25–27] Participants from Bucaramanga who presented within 96 hours of fever onset were eligible for recruitment if they were greater than five years of age. Subjects were excluded based on the presence of the following conditions: history of concomitant diseases such as diabetes, acquired immunodeficiency syndrome (AIDS), hematologic disorders, cancer, or cardiac disease, albuminemia (<3 g/dL), evidence of severe dengue at presentation, for example major bleeding, effusions, or shock. After participants provided informed consent, a physical examination was performed, and a blood sample was obtained to determine hematocrit, albumin levels, and platelet and leukocyte counts. A serum sample was collected and stored at -80C for future biomarker assessment.

Diagnosis of dengue virus infection was confirmed based on viral isolation or serology (seroconversion from a negative to a positive IgM test or a four-fold increase in dengue antibodies in a convalescent blood sample). All study participants were enrolled before the development of DHF/DSS. Participants were followed daily until day 7 of disease with daily microhematocrit measurements to facilitate the recognition of severe dengue. Platelet counts were repeated daily for subjects with previous platelet counts less than 120,000/mm^3 or if there were signs of spontaneous haemorrhage, effusion, oedema or change in hematocrit > 10%. Study participants were classified as DF or DHF/DSS following recovery and full chart review using the 1997 WHO classification system according to the study design.[28]

2.2. Study Design

This study was a case-control study nested within the prospective cohort study (n = 1582) of suspected dengue in Bucaramanga, Colombia. Blood samples were collected from study participants at clinical presentation. Cases were individuals who developed DHF/DSS and controls had dengue fever (DF). Study subjects with available serum samples were eligible for biomarker testing and were randomly chosen from the cohort database using computer generated simple randomization. Serum samples were collected from 15 healthy Bucaramangan adults to derive a population-based normal range.

2.3. Biomarker Assessment

Serum concentrations of biomarkers were measured in samples collected at presentation using ELISA DuoSets from R&D Systems (Minneapolis, MN). Biomarkers measured were C-reactive protein (CRP), CXCL10/IP-10, IL-18 binding protein (IL-18BP), IL-10, chitinase 3-like 1 (CHI3L1), C5a, complement factor D, angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), soluble Tie-1 (sTie-1), soluble Tie-2 (sTie-2), angiopoietin-like protein 3 (Angptl3), angiopoietin-like protein 4 (Angptl4), vascular endothelial growth factor (VEGF), soluble VEGF R1 (sFlt-1), soluble VEGF R2 (sKDR), soluble endoglin (sEng), soluble intercellular adhesion molecule-1 (sICAM-1) and platelet factor 4. All ELISAs were validated prior to use, and sample dilutions were optimized for each biomarker using a dilution curve of serum obtained from febrile subjects with dengue fever in Bucaramanga. ELISAs were tested according to the manufacturer's instructions (R&D Systems) with minor modifications previously described.[29]

2.4. Statistical Analysis

GraphPad Prism v5, SPSS v20 and MedCalc® v12 were used for statistical analysis. Comparisons of continuous variables were performed using the Mann-Whitney U test. Comparisons of proportions were performed using Pearson chi-square test or Fisher's exact test, as appropriate. Exploratory logisitic regression models were built using forward step-wise selection including all variables with $p < 0.10$ by bivariate analysis. Model fit was assessed using the Hosmer-Lemeshow test and ensuring the term was not significant $p > 0.05$. Biomarkers were dichotomized by generating a ROC curve and using the Youden index to identify the optimal cut-off (Youden index: J = max [sensitivity+specificity]-1).

3. Results

3.1. Clinical Characteristics of Subjects that Developed DHF/DSS

111 subjects with microbiologically confirmed dengue virus infection were included in the current study along with 15 healthy controls from Bucaramanga. 65 had DF while 46 developed DHF/DSS over the course of clinical follow up.[3] Individuals that developed DHF/DSS were significantly younger, had higher axillary temperatures and lower mean arterial pressure at presentation and were more likely to report chills, hyperesthesia/hyperalgesia, and dizziness than those with DF (Table 1). The median day of illness at presentation was day 4 for both groups (p = 0.461,