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Evaluation of laboratory tests for dengue diagnosis in clinical specimens from consecutive patients with suspected dengue in Belo Horizonte, Brazil

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ABSTRACT

Background: Dengue is a widely spread arboviral disease in tropical and subtropical regions of the world. Dengue fever presents clinical characteristics similar to other febrile illness. Thus laboratory diagnosis is important for adequate management of the disease.
Objectives: The present study was designed to evaluate the diagnostic performance of real-time PCR and serological methods for dengue in a real epidemic context.
Study design: Clinical data and blood samples were collected from consecutive patients with suspected dengue who attended a primary health care unit in Belo Horizonte, Brazil. Serologic methods and real-time PCR were performed in serum samples to confirm dengue diagnosis.
Results: Among the 181 consecutive patients enrolled in this study with suspected dengue, 146 were considered positive by serological criteria (positive NS1 ELISA and/or anti-dengue IgM ELISA) and 138 were positive by real-time PCR. Clinical criteria were not sufficient for distinguishing between dengue and non-dengue febrile illness. The PCR reaction was pre-optimized using samples from patients with known viral infection. It had similar sensitivity compared to NS1 ELISA (88% and 89%, respectively). We also evaluated three commercial lateral flow immunochromatographic tests for NS1 detection (BIOEASY, BIORAD and PANBIO). All three tests showed high sensitivity (94%, 91% and 81%, respectively) for dengue diagnosis.
Conclusion: According to our results it can be suggested that lateral flow tests for NS1 detection are the most feasible methods for early diagnosis of dengue.

1. Background

Dengue is a worldly common mosquito-borne disease. Next to 2.5 billion people are at risk of infection in the tropical and subtropical regions [1]. Close to 50 million infections occur globally every year [2]. Real prevalence of dengue is probably higher as in less developed countries notification is inefficient and diagnosis confirmation by laboratory assays is not always available. Viral transmission occurs most frequently through the bite of *Aedes aegypti* and *Aedes albopictus* mosquitoes. Four viral serotypes were identified, DENV-1, DENV-2, DENV-3 and DENV-4 [3]. Dengue clinical features vary from an undifferentiated febrile illness to the

Abbreviations: WHO, World Health Organization; DENV, dengue virus; DHF, dengue hemorrhagic fever; BVDV, bovine viral diarrhea virus; PCR, polymerase chain reaction; PPV, positive predictive value; NPV, negative predictive value; SLEV, Saint Louis encephalitis virus; YFV, yellow fever virus.

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severe hemorrhagic form (dengue hemorrhagic fever – DHF) that can lead to shock and death [4,5].

Dengue vector density varies according to rainfall [6]. Therefore, in Brazil, dengue incidence rises seasonally on summer after the raining period [7,8]. The incidence also varies significantly among years in the different regions of the country. From 2009 to 2011, the Department of Health Surveillance of the Ministry of Health of Brazil reported incidences of 205.5, 530.3 and 400.5 per 100,000 persons, respectively [9]. It is clear that dengue is an important health problem in Brazil. Thus, precise and early diagnosis is extremely relevant for adequate management of the disease. Dengue fever is characterized by unspecific symptoms and, in most cases, clinical presentation is similar to other febrile and viral diseases. Thus, clinical criteria are not ideal for the definitive diagnosis of dengue [4,10]. There are many diagnostic tools to detect an acute dengue infection, including virus isolation, RT-PCR and real-time PCR, viral genome sequencing, viral antigen detection and serologic methods [5,11,12]. In Brazil, serology is a common method used in public health services. RT-PCR and NS1 antigen detection by ELISA are sensitive methods for early detection of dengue virus infection [13] but they are not widely used in public health services in Brazil. Moreover, during epidemic periods in areas with high incidence rates, laboratory diagnosis is not always available. In the latter setting, physicians need to rely on clinical and epidemiological criteria to detect possible dengue cases which can lead to false diagnosis and failure to detect other viral pathogens with public health importance [14]. Recently, lateral flow immunochromatographic assays for NS1 antigen detection have been used on primary care services. Those tests are easy to perform, of low comparative cost when adopted for mass-surveys, and convenient for distribution to clinical facilities set far away for main healthcare centers in large countries, like Brazil. We aimed to determine their comparative usefulness in a real clinical epidemic situation.

2. Objectives

In the present work, we analyzed the performance of clinical data, real-time PCR and serologic tests for NS1 and anti-dengue IgM in serum samples from consecutive patients in a primary health care in an endemic area. Belo Horizonte is a large metropolitan area with approximately 5 million people in the Southeast region of Brazil. The aims of the present study were three-fold. Initial experiments were carried out to optimize a real-time PCR reaction against serum samples previously subjected to dengue virus isolation. Second, we evaluated the diagnostic performance of clinical data and real-time PCR for dengue diagnosis in an actual epidemic context. Third, in known dengue positive samples, we compared the diagnostic performance of three lateral flow tests for dengue NS1.

3. Study design

From January 2010 to March 2010, consecutive patients (at the age of 18 or older) with suspected dengue were enrolled in the study after giving written consent to participate. WHO guidelines were used for classifying dengue cases. Clinical data and venous blood samples were collected on the day of admission (acute sample). Six days after illness onset a second blood sample was collected (convalescent sample). According to our case definition, dengue positive cases presented NS1 ELISA and/or anti-dengue IgM ELISA positive tests. Cases with both NS1 ELISA and anti-dengue IgM ELISA negative tests were considered to have other febrile illness as cases with indeterminate result in one of the cited test plus negative result in the other test. Dengue NS1 Ag kit (Bio-Rad Laboratories) was used for NS1 detection in acute samples and anti-dengue IgM was detected in convalescent samples using MAC-ELISA (PanBio Diagnostics, Brisbane, Australia).

In order to evaluate the performance of the real-time PCR protocol we first compared some RNA extraction methods (data not shown). The most reproducible method was obtained by using the QIAamp viral RNA mini kit (Qiagen, Hilden, Germany) as reported before [15]. First-strand cDNA synthesis was performed using MMLV Reverse Transcriptase in standard buffer (Promega, Madison, WI) and reverse primer 5′GGGTCTCCTCTAACCTCTAGTCCT3′. Individual real-time PCR reactions were carried out as previously described by Chien and colleagues [16] in the StepOne™ Real-Time PCR System (Applied Biosystems™). A sensitivity test was performed a priori using known positive samples subjected previously to dengue virus isolation by inoculation in C6/36 *A. albopictus* cells. Dengue virus serotypes were identified as previously described by Lanciotti et al. [17] with slight modifications [16]. The acute sample obtained from the consecutive patients was used for evaluating the performance of the real-time PCR.

Finally, we tested three lateral flow kits for NS1 antigen detection: Bioeasy – Dengue Eden Test Bioeasy (Standard Diagnosis, Pajan-dong, Korea), BIORAD – Dengue NS1 AG Strip (Bio-Rad, Marnes-la-Coquette, France) and PANBIO – Dengue Early Rapid (Inverness Medical, Sinnamon Park, Australia). We used acute samples from consecutive patients presenting positive results in real-time PCR, NS1 ELISA and anti-dengue IgM ELISA. For the specificity test we selected negative acute samples. Statistical analysis was performed in SPSS Statistics 17.0. Categorical and continuous variables where analyzed by Chi-square and Mann–Whitney respectively. A p value equal or smaller than 0.05 was considered statistical significant. Analysis of sensitivity, specificity, PPV, NPV and accuracy were carried out at http://www.openepi.com/.

4. Results

Initial experiments evaluated the sensitivity of the real-time PCR in 58 serum samples previously subjected to dengue virus isolation by culture in C6/36 cells. Serotypes were identified by multiplex PCR: DENV1 (30 samples), DENV2 (20 samples) and DENV3 (8 samples). Real-time PCR detected dengue virus in 52 samples (91% of total). This method also detected DENV4 virus (Fig. 1A).

The specificity of real-time PCR was assessed evaluating culture samples containing other flavivirus (yellow fever virus – YFV-, bovine viral diarrhea virus – BVDV and Saint Louis encephalitis virus – SLEV). Real-time PCR melt curves showed an YFV-unrelated unspecific curve (possibly primer-dimers) and no amplification products with BVDV samples. The amplification products of SLEV virus samples showed two-peak melting curves, in which one peak was similar to that detected for dengue samples (Fig. 1B).

Clinical information and samples for RT-PCR and NS1 ELISA were collected from 181 consecutive patients with suspected dengue on admission. 124 samples were collected for IgM ELISA at the convalescent period from patients that were enrolled in the study on admission and returned at the convalescent period (6 days of symptoms) or patients that were admitted at the convalescent period.

The mean age of subjects was 43 years and 67% were women. According to the case definition used in this study, 81% were confirmed dengue cases (146 patients). The frequency of most clinical and demographic characteristics was equal in patients with acute dengue as compared to non-dengue acute illness. Platelets counts and hematocrit values were similar in both groups (Table 1).

Interestingly, subjects with dengue sought medical attention earlier when compared to non-dengue subjects (3.0 versus 4.2 days). Exanthema was more frequently reported in patients with dengue. Fever, headache, retro-orbitary pain and myalgia were the