Research Article

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Dengue Virus: From Basics to New Technology in Testing & Transfusion Safety

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Abstract

In Puerto Rico, dengue was first recognized in 1915 and the most common recent outbreak occurred in 2010. More than 2.5 billion people (1/3 world's population) live in areas of risk such as Mexico, Central and South America, the Caribbean, and part of Africa and Asia continents. The Dengue exists in United States in the southern areas as well as in the Mexico border. An estimated 50 million cases occur annually and DENV was the leading cause of febrile illness among 17,353 ill travelers returning from the Caribbean, South America, South Central Asia, and Southern Asia.

Dengue fever is caused by the transmission of four dengue virus types to humans primarily via a mosquito vector. Most people infected with the virus have no symptoms or a mild fever and transfusion-transmitted dengue infections have been described in three clusters (Hong Kong, Singapore and Puerto Rico). Based on the data generated during the nonstructural protein 1 (NS1) antigen (Ag) Investigational New Drug (IND) by a research transcription-mediated amplifications (TMA) assay, the sensitivity of the Dengue TMA test is at least 2 to 3 fold higher than NS1 Ag assay. Dengue RNA detected by TMA is detected prior to NS1 Ag in infected individuals and persists for longer periods of time during the ramp up phase of antibody production when donors may still be infectious. Donations during the window period between TMA and NS1 Ag detection may be infectious. Transfusion transmission has been demonstrated. Only 17.5% of 140 People from PR studied by a questionnaire know that mode of transmission. We need to educate more health professionals. We still need more studies in testing and prevention.

Keywords: Dengue Virus; Testing; Transfusion safety

Abbreviations: NS1: Nonstructural Protein 1; Ag: Antigen; IND: Investigational New Drug; TMA: Transcription-Mediated Amplifications; RNA: Ribonucleic Acid; PR: Puerto Rico; ARC: American Red Cross; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus; WNV: West Nile Virus; DENV: Dengue Virus; CDC: Center for Disease Control; RBC: Red Blood Cells; AABB: American Association of Blood Banks; US: United States; (+): positive; NAT: Nucleic Acid Test; FDA: Food and Drug Administration; PCR: Polymerase Chain Reaction; IgM: Immunoglobulin M; DHF: Dengue Hemorrhagic Fever; RR: Repeat Reactive; IR: Initial Reactive; CP: Confirmed Positive; ARRA GO: American Recovery and Reinvestment Act Grants Opportunity; BSRI: Blood System Research Institute; PBMC: Peripheral Blood Mononuclear Cell

Introduction

We know that Hepatitis B & C, Human Immunodeficiency Virus, Human T-cell Leukemia/Lymphoma Virus-I & II, and West Nile Virus (WNV) as well as Babesia and Chagas Disease are frequent infectious agents transmitted by blood transfusion, but there is not enough knowledge about Dengue Virus [1]. A questionnaire of 15 questions to 140 persons was administered from September to November of 2012. 88% were adults, 100 women and 40 males. Most of them were from Caguas, Carolina, San Juan and Bayamón. 60% were professionals. 100% have good knowledge of dengue and 90% know the mosquito. 77% were aware of the actual epidemics, but males are 10% more knowledgeable than women. 47% is not prepared to fight against the Dengue. 66% didn't know that PR has a CDC office for Dengue. Only the 17.5% have knowledge that Dengue Virus (DENV) can be transmitted through a blood transfusion [2]. There is a need for community outreach and educational program for dengue prevention and in particularly about transfusion-related transmission.

Dengue is an infection caused by an arthropod-borne virus, in particular, by four related RNA viruses of the genus Flavivirus, dengue virus (DENV) -1,-2,-3, and -4. The mosquito *Aedes aegypti* is the principal vector. DENV's are transmitted from person to person and humans are the main amplifying host. The disease spectrum goes from a mild acute febrile illness to an hemorrhagic fever and severe shock [3].

In Singapore [4] a donor was symptomatic one day after donation. Two recipients (Red Blood Cells, Fresh Frozen Plasma) developed dengue-related illness and seroconverted. Third recipient (platelet) asymptomatic but developed Immunoglobulin M and G antibodies. Donor and two symptomatic recipients were positive for DENV-2 RNA. In Hong Kong [5], one donor symptomatic one day after donation and one recipient (RBC) developed dengue-related illness and seroconverted. The donor and recipient were positive for DENV-1 RNA. In Puerto Rico [6] a 6-years-old child symptomatic with DENV-4 isolated from blood and tissues 4 days post bone marrow transplant. Few cases of transfusion-transmitted dengue have been reported in the literature, and the true incidence of transfusion-transmitted dengue is unknown because there is no surveillance for such events and if

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Received August 29, 2013; **Accepted** October 03, 2013; **Published** October 06, 2013

Citation: Morales Borges RH (2013) Dengue Virus: From Basics to New Technology in Testing & Transfusion Safety. J Blood Disorders Transf 4: 161. doi: [10.4172/2155-9864.1000161](https://doi.org/10.4172/2155-9864.1000161)

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a case is suspected, it is difficult to prove transfusion transmission (versus vector-borne transmission) in recipients from dengue endemic countries. Nevertheless, transfusion risk models and assessments of viremia prevalence among blood donations indicate the potential for high transfusion transmission of DENV in dengue endemic areas and the American Association of Blood Banks (AABB) Transfusion Transmitted Diseases Committee recently identified DENV as one of three high priority infectious agents with actual or potential risk of transfusion transmission in the US [1].

One study in PR by American Red Cross from September to December 2005 using TMA in all blood donors, they found that 12 (0.07%) of 16,521 blood donations tested were TMA-positive, furthermore, live virus was recovered from three of the 12 TMA (+) donations, indicating that at least 3 were capable of transmitting infection to recipients [7].

The prevalence of dengue viral nucleic acid in blood donations in that study was similar to that estimated for WNV in the areas experiencing outbreaks in the continental United States in 2002 before universal screening using mini pool nucleic acid test (NAT) was implemented in July 2003. ARC recommended further evaluation to assess the risk of dengue transmission by TMA (+) donations and the cost and benefit of routine dengue screening in endemic regions.

Methods

We reviewed the medical literature published through PUBMED across the years as well as the data from the studies performed by the American Red Cross Biomedical Services for analysis. Before the results, we want to explain the technology of the most common Dengue testing available.

Dengue TMA assay

It is a research assay based on same technology as FDA licensed PROCLEIX[®] blood screening assays (HIV-1/HCV Assay, ULTRIO[®] Assay, and WNV Assay) and is a qualitative NAT for the detection of dengue virus RNA [8]. Target capture is by transcription-mediated amplification (TMA) seen as chemiluminescent detection. Design most closely resembles the PROCLEIX WNV Assay. Same base reagent formulations with dengue-specific oligonucleotides. Currently uses the same TIGRIS software, with all cutoff calculations and validity criteria identical to WNV Assay. Runs on fully automated TIGRIS[®] System [9]. It provides 1000 results in 14 hours, time to first result in 3 hours and 38 minutes, followed by ~100 results/hour, and Process controls for all assay steps

Dengue NS1 Ag detection

The full name is Platelia Dengue NS1 Ag assay [10] and it is a test made by Bio-Rad Laboratories and Pasteur Institute, introduced in 2006. The test allows rapid detection on the first day of fever, before antibodies appear some 5 or more days later and has been adopted for use in some 40 nations. The method of detection is through PCR. India introduced that test in 2010. Accurate diagnosis of dengue can be achieved with NS1 Ag detection. NS1 gene is highly conserved among dengue viruses. NS1 Ag is highly specific (no cross-reactivity with other flaviviruses). It correlates with viral replication.

Results

All donations made between 20 Sept and 4 Dec 2005 were tested for the presence of dengue viral nucleic acid. The Epi Curve above

has number of cases on the y-axis, and week of symptom onset on the x-axis. Suspected cases are in yellow, and confirmed cases in red. The number of cases began rising in August with a peak in mid-September.

The study period, highlighted above, began one week after the peak and ended 2 weeks before the end of the year. This study period was intentionally chosen during the high dengue transmission season to increase the yield of dengue positive donors. 8684 dengue RNA negative samples retested using a more sensitive alternative TMA assay and the results were: Prevalence of 1:529 (0.19%) and Specificity 15,314/15,321 for 99.95% [11]

2007 dengue season in PR [7]

10,508 cases or 2.9 cases per 1000: All 4 DENV serotypes in circulation. First year that all 4 serotypes reported since 1998—first year all 4 serotypes ever reported. More than 50% were hospitalized, 1/3rd reported hemorrhage, 2.2% had DHF, and 44 deaths. ARC donor samples were retained in a linked repository for TMA testing (recipient tracing). Testing split into 2 parts: samples representing units issued in the continental US vs. PR.

- RR needs further testing at CDC PR
- 28 IR/25 RRs equals to 1:614 RR (0.16%)
- 15,321 tested equals to 99.98% specificity
- 14/25 (+) at 1:16 (56%)
- 11/25 RNA titers of 105 – 109 (44%)
- All 11 infected mosquito cell cultures
- 9/11 detected at 1:16
- 6/22 IgM (+) (27%)
- 2/6 quantifiable virus
- DENV-1, 2 and 3 detected

Dengue RNA prevalence rates in Puerto Rico [7,12]

2005 prevalence (testing ARC repository)

- Testing performed by NAT using research dengue TMA assay.
- RR goes to Dengue Branch, CDC for serotype-specific PCR (qualitative/quantitative); IgM and mosquito cell culture
- No donor/recipient notification (unlinked)
- Middle/end of epidemic season
- 12 total dengue RNA (+) of 16,521 tested
- 1:1376 (0.073%)
- 12/14 samples RR equals to 99.99% specificity
- 5/12 (42%) reactive in a MP of 16
- 4/12 (33%) PCR positive; 2×10^3 – 8×10^7 copies/mL.

American Red Cross initiated an IND study in PR using Bio-Rad NS1 Ag assay early in 2010, which was linked to a 2009 ARRA GO award with BSRI. They enrolled 75–100 donors in long-term follow-up, generated systematic data on viral and immune parameters, and created a plasma/PBMC repository.

The yield of NS1 Ag results from March 2010 to August 2012 was disappointing. It detects only high-titer, RNA positive donations

during acute infection prior the development of antibodies. 50% yielded infectious virions and only 9 positives were identified in 2010. Retrospective NAT (TMA) had a 10-fold higher yield. No positives

found in 2011 as non-outbreak year. A prospective TMA screening was introduced under IND in PR on August 2012 (Figures 1-9).

Figure 7: 2012-2013 Dengue in PR TMA R Donors (02/22/2013).

Figure 8: 2012-2013 Summary of TMA Reactive Donations.

Figure 9: 2012-2013 Summary of TMA Donor Follow-Up.

Discussion

We noticed that the cases are lower than in previous years and they are lower after the storm/hurricane season. We are concern about the

false positive results with NS1. Can we obtain a more sensitive test than NS1? The Hospitals do not have an idea of how big is this problem regarding transfusion transmission, but they are collaborating so far. They don't want to make this a big issue because they don't want to develop a risk management case (lawsuit?) from the patient's side. Many physicians are unaware of dengue transfusion transmission. Are we responsible of giving them the appropriate education about this issue? How we can get them involved in prevention of transfusion transmission?

Prevention of Transfusion-Associated Transmission during epidemic periods involved to discontinue imports from PR into the Continental US during dengue outbreaks (Occurred May 18, 2009 for the ARC), defer 120 days from dengue diagnosis or onset of illness whichever is later, implement enhanced post-donation information, and recognizing that 53-87% are asymptomatic. We need to assess the risk of dengue transmission by TMA-positive donations & NS1 Ag (+) as well as the cost-effectiveness of routine dengue screening. Look for more sensitive test. Evaluate the Weather seasons/patterns and Aedes Mosquito Monitoring in Puerto Rico and the correlation with the prevalence and transmission rate to determine when is better to do blood drives in the Island. Defer at-risk donors, e.g. symptoms of fever, travel history to endemic regions, exposure to dengue patients, etc. Continue with the Dengue Follow-Up Study. Track & Receive Plasma Units associated with Reactive Samples for Confirmatory Testing [13].

Conclusion

Transfusion transmission of DENV has been demonstrated. We need to educate more the health professionals (e.g. Nurses, Physicians). There are few options for minimizing dengue risk in the blood supply, but we still need more studies in testing and prevention. A guideline for these cases needs to be established. Since the actual risk of dengue disease following transfusion is not known, combine efforts have been gather on this "look back" study in which we intend to determine the risk of dengue among persons who received blood products that later tested positive for DENV by molecular diagnostic testing. This study would estimate rates of dengue and possibly transfusion-transmitted DENV infection among recipients of DENV-positive donations. These rates would be compared to estimate rates as determined by infection transmission models. Given that the epidemiology of dengue in Puerto Rico is similar to that of other dengue endemic countries, findings from this study can be used to help improve the safety of the blood supply worldwide. The CDC, FDA, ARC and other blood banking groups would use these findings to make blood safety recommendations and the need for routine DENV tests for donors residing in dengue endemic areas to prevent transfusion-transmitted dengue. This study has been approved by both ARC and CDC-DB Institution Review Boards (IRBs) and we have yet started requesting Hospitals and Physicians collaboration. Associated Researcher has started the process of data collection for this important research. We appreciate and thank you for your commitment with the Public Health of our country.

Acknowledgment

I thank Susan L. Stramer, Ph.D. (Executive Scientific Officer, Scientific Support Office, ARC, Biomedical Services), Antonio de Vera, BSE, PE, MS (Executive Director, ARC of PR, Blood Services), Carmen Merced, MT (Manager, Donor Information, ARC of PR, Blood Services), Desiree Matos, RN, BSN (Research Associate, Dengue Study-ARC of PR & CDC-Dengue Branch), and Aidsa Rivera, MS (Epidemiologist/Surveillance Officer, CDC, Health Department of PR).

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