



# Virological and Serological Markers in Dengue Patients from Venezuela and Nicaragua

Didye Ruiz<sup>1\*</sup>, Susana Vázquez<sup>1</sup>, Elci Villegas<sup>2</sup>, Angel Balmaseda<sup>3</sup>, Maritza Pupo<sup>1</sup>, Mayling Alvarez<sup>1</sup>, Delfina Rosario<sup>1</sup>, Herminia Bendezu<sup>2</sup>, Naifi Calzada<sup>1</sup>, Maria G. Guzman<sup>1</sup>

<sup>1</sup>"Pedro Kouri" Tropical Medicine Institute, PAHO/WHO Collaborating Center for the Study of Dengue and Its Vector, Havana City, Cuba

<sup>2</sup>"Jose W Torrealba" Experimental Institute, Los Andes Trujillo University, Bogota, Venezuela

<sup>3</sup>CNDR, Managua, Nicaragua

Email: \*didye.ruiz@ipk.sld.cu

**How to cite this paper:** Ruiz, D., Vázquez, S., Villegas, E., Balmaseda, A., Pupo, M., Alvarez, M., Rosario, D., Bendezu, H., Calzada, N. and Guzman, M.G. (2017) Virological and Serological Markers in Dengue Patients from Venezuela and Nicaragua. *Open Access Library Journal*, 4: e3309. <https://doi.org/10.4236/oalib.1103309>

**Received:** February 16, 2017

**Accepted:** April 9, 2017

**Published:** April 12, 2017

Copyright © 2017 by authors and Open Access Library Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

## Abstract

Latest estimates suggest 390 million infections of dengue occur each year, of which 100 million results in symptomatic disease. The new clinical classification proposed by WHO-TRD 2009 simplifies dengue fever (DF) into dengue (D) and severe dengue (SD) with an intermediary group of "dengue with warning signs" (DWS). Early, sensitive and specific diagnosis of dengue is needed for appropriate patient management as well as for early epidemic detection. Virological and serological markers in sera from dengue confirmed patients from Venezuela and Nicaragua were evaluated. A total of 232 sera collected in the acute (days 1 to 5 of fever onset) and convalescent (days 7 to 14) phases of illness were tested by Platelia NS1 antigen ELISA and IgM, IgA and IgE capture ELISA methods. NS1 was detected with highest OD values in the acute phase for primary and secondary cases. IgM was detected in primary and secondary groups but the highest OD figures were observed after defervescence day. IgA and IgE detection were almost absent for primary cases, contrary to secondary cases which showed higher values but after defervescence day. NS1 could be used as effective diagnostic marker in the acute phase of illness for primary and secondary dengue infections. IgM detection is still an invaluable tool for routine dengue diagnosis but delay. IgA detection could define secondary infection in early phase of illness. IgA/IgE could be evaluated as prognostic markers associated to SD cases.

## Subject Areas

Immunology, Infectious Diseases

## Keywords

Dengue Infections, IgM, IgA, IgE, NS1 Protein

## 1. Introduction

Dengue is the most important mosquito-borne viral disease of humans and an enormous public health burden in affected countries. Latest estimates suggest 390 million infections of dengue occur each year, of which 100 million results in symptomatic disease [1].

Dengue viruses (DEN) belong to the family *Flaviviridae*, genus *Flavivirus*. There are four dengue virus serotypes (1, 2, 3, and 4), which are responsible for this disease. Dengue infection can range from asymptomatic infections, undifferentiated fever and classical dengue fever (DF) to severe forms of the disease: dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2] [3]. The new clinical criteria of dengue case classification (dengue and severe dengue, WHO 2009) provide practical guidance and show a better categorization in accordance with disease severity [WHO/TDR. 2009].

The laboratory diagnosis of DEN is based on virus isolation, viral RNA detection, or detection of DEN-specific IgM and IgG serum antibodies [4] [5] [6]. However, all these assays have limitations: Virus isolation requires lengthy culture, is expensive and has relatively poor sensitivity; RT-PCR is accurate and ideal for early diagnosis but requires specialised equipment not always available in tropical settings where many flaviviruses predominate; serological approaches can give results in few hours or minute but antibodies detection is not available early in course of the disease, moreover, the problems with antigenic cross-reactivity of IgG than IgM particularly in regions of the world where more than one flavivirus co-circulates [7].

An early and accurate diagnosis of dengue can assist in patient management by directing clinical attention to the appearance of major warning signs of severe or even life threatening complications; it can facilitate vector control activities in the community and prevents unnecessary antibiotic usage [8]. For that reason, the studies of other virological and serological markers as NS1 protein, IgA and IgE have been made based in their usefulness for an early dengue diagnosis [7] [9] [10].

The DENCO project was designed for producing new evidences through basic and implementation research for more effective dengue prevention and case management by reducing viral transmission and case fatality. As part of this project we have evaluated the virological (NS1 protein) and serological (IgM, IgA and IgE) responses in serum samples collected from patients of Venezuela and Nicaragua with a confirmed dengue infection.

## 2. Material and Methods

### 2.1. DENCO Study

The DENCO (Dengue Control) project was a multi-centre prospective observational study of dengue in Southeast Asia (Malaysia, Thailand, The Philippines and Viet Nam) and the Americas (Nicaragua and Venezuela). The study sites at which patients were enrolled for this particular investigation were: Hospital Manuel Jesus de Rivera, Managua, Nicaragua; Research Centre Jose W. Torreal-

ba, Los Andes Trujillo University, Bogota, and Hospital Central, Maracay, Venezuela.

## 2.2. Patient Enrolment

Venezuela and Nicaragua patients above 6 months of age, with clinically suspected dengue and fever for less than 7 days were enrolled in the study. In case of children were written an informed consent by the study participant, or a parent/guardian. Out-patients and in-patients were recruited at 5 centres. Patients were followed by trained study physicians using standardised case report forms (CRFs) describing clinical, laboratory, diagnostic and management information in detail. Ethical approval was obtained from the Ethics Review Committee of WHO and each institution involved. Two or three samples were collected for each patient, during the acute (days 1 to 5 of fever onset) and convalescent (days 7 to 14) phases. For each patient was determinate the day of defervescence, defined as day 0. Dengue diagnosis was confirmed by virological (virus isolation and/or RT-PCR detection) and/or serological methods (IgM and/or IgG) [12] [13] [14]. The patients were classified according to the type of infection (primary or secondary) and following the WHO criteria for the clinical classification of dengue (D) and severe dengue (SD) [15] [16]. The NS1 detection and IgM, IgA, IgE responses were studied in the tested samples.

## 2.3. Characteristics of the Study Population

Between August 2006 and May 2007 a total of 2259 patients were recruited to the DENCO study at the 11 participating hospitals. NS1 detection was attempted using at least one of the two NS1 tests in 1821 patients. From amongst the 1821 patients, there were 1385 with laboratory-confirmed dengue (**Dataset for analysis**) and 45 with no laboratory evidence of acute or recent dengue [11]. In this work, we use only serum samples from Venezuela and Nicaragua patients.

## 2.4. Laboratory Investigations

Serological and virological dengue diagnostics were performed in each participating country according to local protocols, with support provided by WHO designated laboratories as necessary. The definitions employed for “**confirmed dengue case**” was RT-PCR positive or virus isolation positive and IgM seroconversion (paired samples) and IgG seroconversion or fourfold or greater increase in titer (paired samples). All tests processed by Managua and Maracay Hospitals were repeated at IPK, Cuba. The study was enrolled 71 patients from Venezuela and 23 from Nicaragua.

## 2.5. Virus and Antigens

Dengue antigens used in the serological studies were obtained from infected suckling mice brain extracted by the sucrose-acetone method [17]. Reference

dengue viruses strains were employed: dengue 1 (Hawaii strain), dengue 2 (New Guinea C strain), dengue 3 (H-87 strain) and dengue 4 (H-241 strain).

## 2.6. ELISA Tests

In-house ELISA tests were used with the conditions previously established: IgM, IgA and IgE antibody capture immunoassays (MAC-ELISA, AAC-ELISA, EAC-ELISA) and ELISA Inhibition Method (EIM) for IgG antibody detection [13] [14]. A serum sample was considered positive by MAC and AAC-ELISA when the optical density ratio (OD ratio) was  $\geq 2$  and for EAC-ELISA test  $\geq 1.4$ . This value was calculated as P/N where P represents the OD of each serum sample and N represents the mean OD of the negative control wells. By EIM the inhibition percentage was calculated as: Inhibition % =  $[1 - (\text{OD sample} / \text{OD negative control})] \times 100$ . The antibody titer of each serum was considered as the highest dilution (between 20 and 10240) with a percentage of inhibition  $\geq 50$ . A serum with a percentage of inhibition  $< 50$  was considered negative for IgG dengue antibodies ( $< 20$ ). Platelia Dengue NS1 AG ELISA (BioRad, France) for the detection of NS1 protein was performed according to the manufacturer's recommendations. Sample result is expressed by Ratio using the following formula: Sample Ratio = S/CO, where S is the OD obtained in the sample and CO is the cut-off value corresponds to the mean value of duplicate OD. A sample ratio  $< 0.5$  corresponds to negative;  $0.5 \leq \text{ratio} < 1.0$  is equivocal and  $\geq 1.0$  is positive.

## 2.7. Statistical Analysis

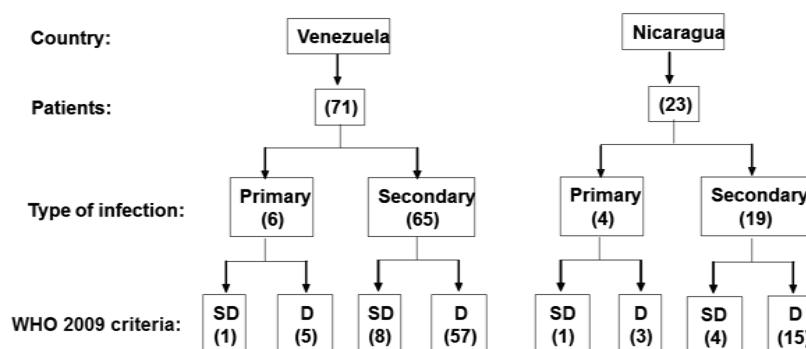
Original data analysis was performed at the Section of Clinical Tropical Medicine at the University of Heidelberg, Germany, using STATA versions 9.2 and 10, (STATA corporation, College Park, Texas) [11]. To determine IgM, IgA, IgE, NS1 OD mean ratios and mean of defervescence days with confidence interval (CI) 95% in primary and secondary cases was employed GraphPad Prism 5 program (2007). Also, Excel program 2007 was used for figures.

## 3. Results

### 3.1. Venezuela and Nicaragua Patients' Classification

In the study were enrolled 71 patients from Venezuela and 23 from Nicaragua. These patients were classified according to the type of infection in 10 primary cases and 84 secondary cases. Also, according to the WHO dengue clinical classification, 80 patients were classified as D and 14 as SD (**Figure 1**). In the Venezuelan group 6 (8.5%) patients were classified as primary and 65 (91.5%) as secondary cases; 62 cases were classified as D and 9 as SD cases. In the Nicaraguan group 4 (17.4%) patients were classified as primary and 19 (82.6%) as secondary cases, with 18 as D and 5 as SD cases.

All primary and secondary cases were analysed together independently of the origin country due to the small number of primary D/SD samples for the study.



**Figure 1.** Venezuelan and Nicaraguan dengue patients classified by infection type and WHO 2009 clinical criteria [Dengue (D) and Dengue severe (SD)].

### 3.2. Kinetics of NS1, IgM, IgA and IgE in Serum Samples from Primary and Secondary D Cases

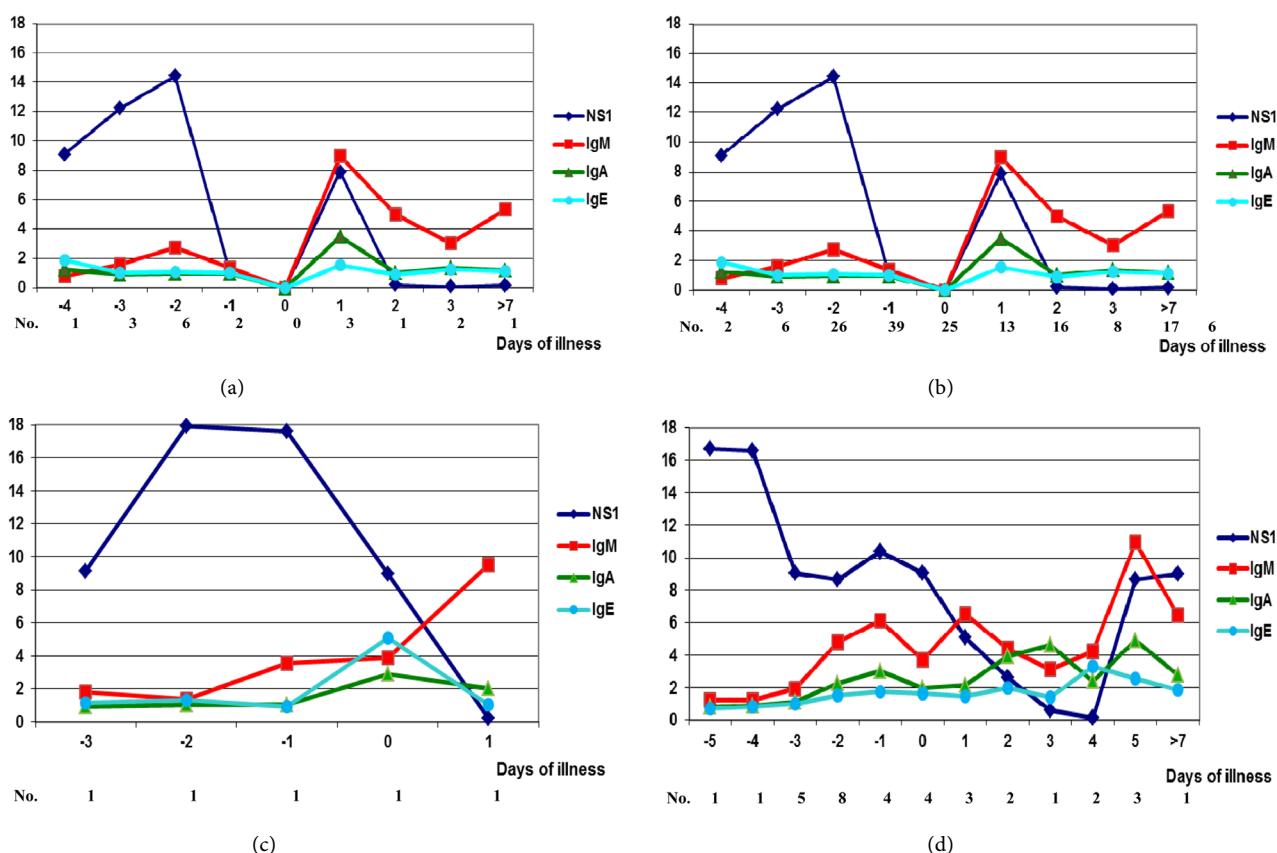
The detection of the different markers in primary and secondary D cases was analysed in relation to the day of defervescence (day 0) (**Figure 2**). The mean of defervescence day in primary and secondary D cases was 5.00 (CI 95%: 3.42 - 6.58) and 5.34 (CI 95%: 5.06 - 5.66) respectively.

In primary cases (**Figure 2(A)**), NS1 was detected from day -4 to day 1, with the highest OD mean values before day 0. Specific IgM was detected from day -2 although higher positivity was observed after day 0. Specific IgA only was detected at day 1 in one convalescent serum sample and specific IgE was not detected for primary D cases. In secondary cases (**Figure 2(B)**), NS1 protein was detected as early as day -5 with the highest OD mean ratio on this day. Also a decrease toward day 2 was observed in relation with the increase of the IgM response. However, positive OD mean ratios in relation to three positive convalescent samples were observed at days 6 and 7 after day 0. A progressive increase of IgM antibody was observed from day -4, with a similar pattern for IgA and IgE but starting at day -2. In this group, the highest OD values were observed in samples collected after day 0 for all serological markers.

### 3.3. Kinetics of NS1, IgM, IgA and IgE in Serum Samples from Primary and Secondary SD Cases

The mean of defervescence day in primary and secondary SD cases was 6.50 (CI 95%: 0.17 - 12.85) and 5.90 (CI 95%: 5.21 - 6.64) respectively.

Two SD patients developed a primary infection. From them, five serum samples were collected. NS1 was detected as early as day -3 and kept in detectable levels until day of defervescence (**Figure 2(c)**). NS1 decreased as IgM increased. IgM was detected at day -1 with a trend to increase OD for samples collected after day 0. IgA and IgE levels were almost absent, being detected only in two samples. In secondary SD cases (**Figure 2(d)**), levels of NS1 protein decreased in time and showed highest values in days before defervescence. However, serum samples with NS1 at days 5, 6 and >7 were still detected. The serological markers (IgM, IgA and IgE) revealed a tendency to increase OD in time with highest val-



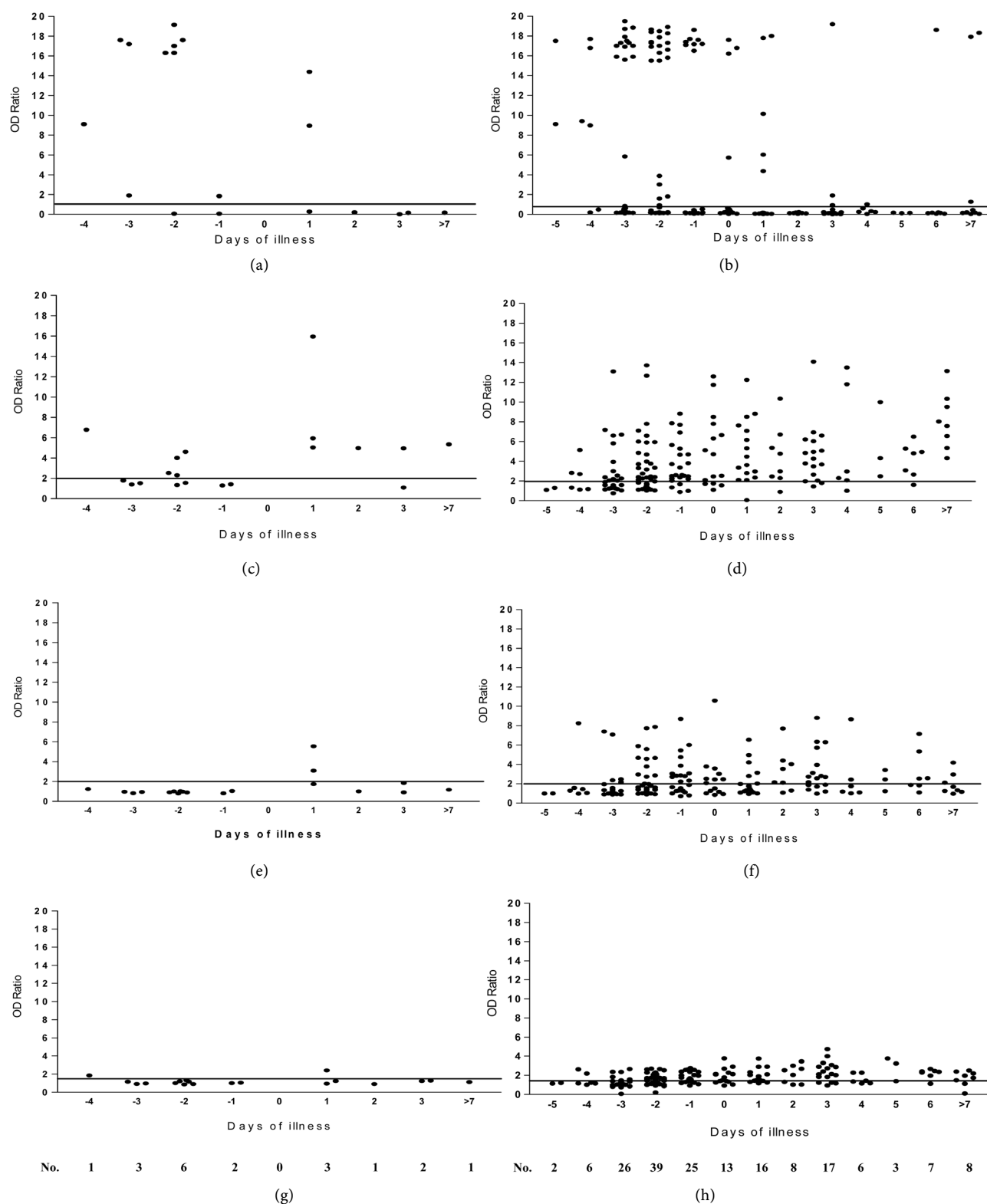
**Figure 2.** Kinetics of NS1, IgM, IgA and IgE from serum samples of primary and secondary D/SD cases in relation with defervescence day (day 0). (a) primary (d) (b) Secondary (d) (c) Primary DS and (d) Secondary SD. The principal y-axis shows the optical density (OD) mean ratio.

ues in days after defervescence. IgM was detected from day –3 and IgA and IgE at day –2.

### 3.4. Levels of NS1, IgM, IgA and IgE Serum Samples from Primary and Secondary D Cases

Levels of NS1, IgM, IgA and IgE in serum samples from D patients with primary and secondary infection are shown in **Figure 3**.

In primary cases, highest NS1 OD and positive percentage (83.3%) values in days before defervescence were showed. (**Figure 3(a)**). After day 0, the positivity was almost absent (29%) with detected levels until day 1. Levels of IgM antibodies begin to be detectable in day before day 0 but highest positive percentage (86.0 %) was obtained in days after defervescence with a tendency to increase at the time (**Figure 3(c)**). IgA and IgE levels were almost absent in primary cases (**Figure 3(e)** and **Figure 3(g)**), with poor percentages of 29.0% and 14.3% respectively after defervescence. In the same way, secondary cases showed highest positive percentage (51%) in days before defervescence decreasing at the time. Levels of NS1 remain detected until day >7, representing collected samples in the convalescence phase of illness (around day 10 or more of fever onset) (**Figure 3(b)**). For the serological markers, highest percentages of 89.2 (IgM), 52.3 (IgA)



**Figure 3.** Levels of NS1, IgM, IgA and IgE from serum samples of primary and secondary D cases in relation with defervescence day (day 0). (a) (c) (e) (g) NS1, IgM, IgA and IgE for primary (d) cases; (b) (d) (f) (h) NS1, IgM, IgA and IgE for secondary D.

and 72.3 (IgE) in samples collected after defervescence were found (**Figure 3(d)**, **Figure 3(f)** and **Figure 3(h)**).



### 3.5. Levels of NS1, IgM, IgA and IgE Serum Samples from Primary and Secondary SD Cases

Levels of NS1, IgM, IgA and IgE in serum samples from SD patients with primary and secondary infection are shown **Figure 4**. In primary SD cases, the tendency for NS1 protein levels is to increase the highest level (100%) in days before defervescence contrary to the serological markers, which showed a rise at time although with a low positivity for IgA and IgE (**Figure 4(c)**, **Figure 4(e)** and **Figure 4(g)**). For secondary SD cases, highest percentage (63.2%) was observed before day 0 and a persistence of NS1 protein similar to secondary D cases was detected (**Figure 4(b)**). The serological responses were also similar to secondary D cases (**Figure 3(d)**, **Figure 3(f)** and **Figure 3(h)**).

## 4. Discussion

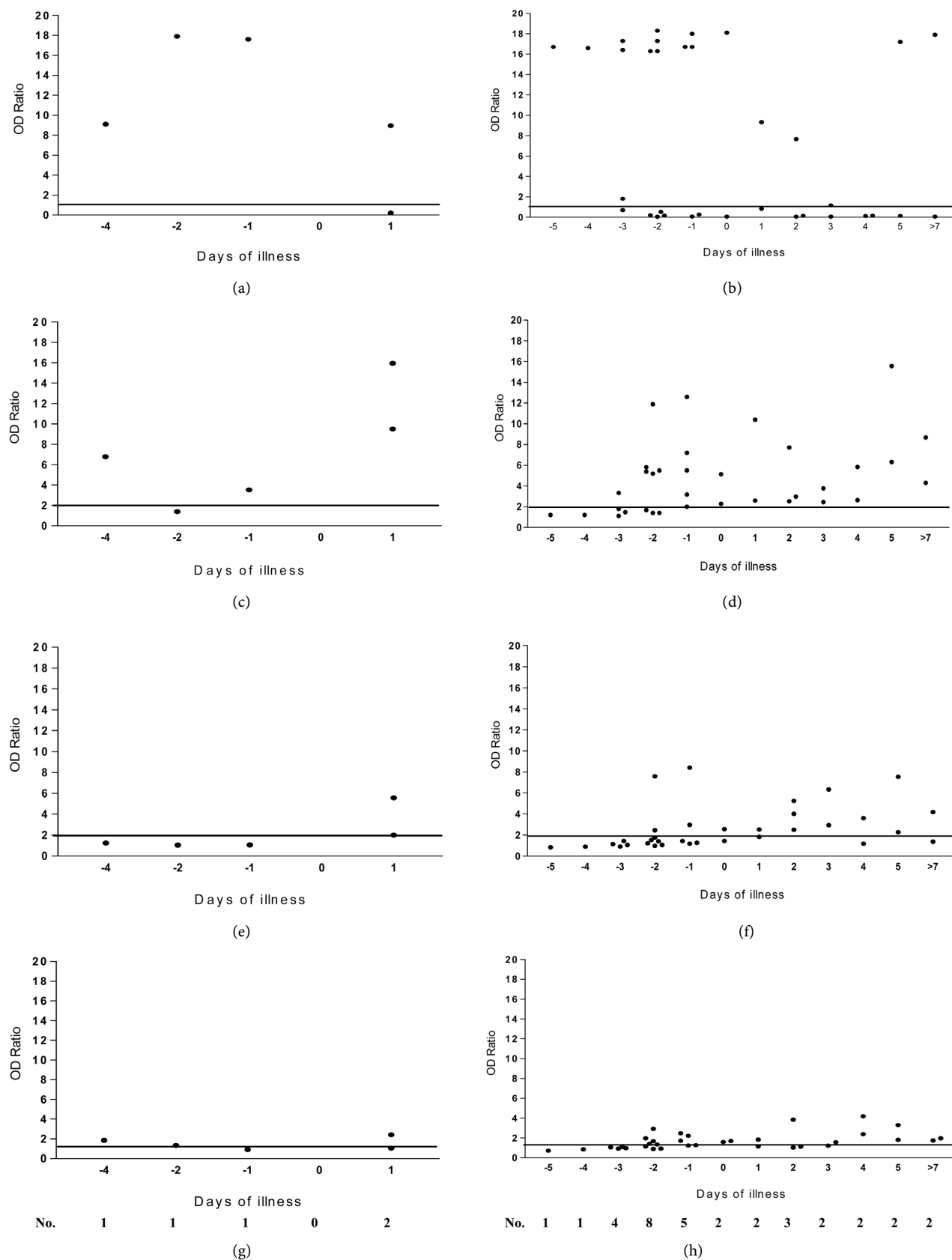
For dengue disease, fever and other general complaints are considered as early clinical manifestations between first and second day of the disease and only after the third day defervescence are observed and with great importance for severe cases [18].

NS1 is a highly conserved glycoprotein that exists in multiple oligomeric forms and is found at different cellular locations: either cell-membrane-associated (mNS1), in vesicular compartments within the cell or on the cell surface, and as a secreted lipid-rich, extracellular (nonvirion) species [19]. Recently, NS1 has been proposed as an early marker of dengue infection. ELISAs for NS1 antigen detection have demonstrated the presence of high concentrations in acute sera from dengue patients [7] [20] [21], coinciding the viremic and febrile periods [22] [23].

In the present work, early detectable NS1 ratios from primary and secondary dengue patients were showed, independently of the clinical picture. The best period of NS1 detection was in days before defervescence day (between days 0 and 4). The non-detectable NS1 levels toward day 2 after defervescence could be related to the production of anti-NS1 antibodies that may form immune complexes and target epitopes are not accessible to either the plate-bound or probe mAb in the NS1 ELISA. However, in some samples from secondary cases, NS1 remain detectable at day 10 or more of onset of fever. Alcon *et al.* (2002), Shu *et al.* (2003 and Dussart *et al.* (2006) [24] [25] [26] are in agreement about the detection of NS1 antigen until days 8 and 9 of onset while [27] [28] found detectable NS1 until days 14 and 18 of onset.

Primary dengue infection is characterized by an increase in the level of dengue-specific IgM antibody 3 - 5 days after the onset of symptoms, accompanied by low levels of dengue-specific IgG antibodies. In a secondary infection, dengue-specific IgM antibody can be present, whereas IgG increases rapidly to very high levels at days 1 to 2 after onset of symptoms [5] [29]. Here, IgM levels start to increase earlier in secondary than primary D cases. For severe cases, the IgM responses were analogous to D cases. Highest OD mean ratios after defervescence always were showed. Although a variable and sometimes absent IgM res-





**Figure 4.** Levels of NS1, IgM, IgA and IgE from serum samples of primary and secondary SD cases in relation with defervescence day (day 0). (a) (c) (e) G: NS1, IgM, IgA and IgE for primary SD cases; (b) (d) (f) H: NS1, IgM, IgA and IgE for secondary SD.

ponse has been reported in secondary dengue infections [30] [31] [32], we don't observed this behaviour. Memory B cell could be playing an important role in the secondary dengue immune response. Generally, memory B cells response has been associated to the IgG release and not to IgM. B cells expressing CD27 molecule on their surface and undergo somatic hypermutation in immunoglobulin (Ig) variable (V)-region genes, generate Igs rapidly and vigorously in the secondary immune response [33] [34]. Some authors have described the presence of a population of memory B cells (IgM<sup>+</sup>IgD<sup>+</sup>CD27<sup>+</sup>) in variable amounts into the memory B cell pool of human peripheral blood [33] [35] [36] and have been announcement its capacity to produce a high-affinity IgM antibody following stimulation [37] [38]. High homology between dengue serotypes and recognition of common epitopes, during a sequential could explain the IgM levels detected in our cases.

Dengue IgA antibody detection in serum samples from individuals with dengue infections have been proposed as a serological marker for diagnosis [9] [39] and as an indicator of recent infection [40] [41]. In this study, highest IgA ratios were observed for secondary D and SD cases and mainly in samples collected after defervescence day. In all primary cases the responses were absent in days before day 0. The IgA has been commented with an analogous behaviour to IgG antibody. In primary infection must be detectable after the release of IgM, while in secondary infection IgA appear early in acute phase by the common epitopes recognition by memory B cell clones circulating on blood [40] [42]. The induction of high-rate IgA levels requires a group of factors which must act synergistically in the Ig production predominantly toward IgA isotype: engagement of CD40 (B cells) by CD40 ligand (CD40L, Ag-activated CD4<sup>+</sup> T cells) which determines class switch gene recombination; TGF- $\beta$  secreted by CD40L-induced B cells is required for switching to IgA; the presence of IL-10 increasing both the number of IgA lymphocytes and the amount of secreted IgA. [43] demonstrated higher serum levels of IL-10 in dengue patients associated with secondary infection and [44] suggested an important role of TGF- $\beta$  and IL-10 mediators as regulatory and anti-inflammatory cytokines, associating these presences with protection in dengue secondary infection. Therefore, anti-dengue IgA detection in serum samples could be evaluated as an alternative diagnostic for secondary dengue case definition in early phase of infection (between days 0 and 4).

IgE antibody may play a protective role in some parasitic, bacterial and viral infections in humans, and possess anti-tumor properties in vitro [45]. Some authors have described IgE antibodies elevation in patients with dengue infections. [46] found both total and specific increased of IgE levels in patients with DHF, [47] observed higher levels of total IgE in dengue patients contrary to control group (subjects with no exposure); [48] observed higher levels of specific IgE in DHF/DSS cases than DF cases and healthy individuals; [13] found significant differences of IgE OD ratios between primary and secondary dengue cases, observing the higher values for the last group. Similar to IgA response, IgE OD ratios were detectable in secondary D and SD infection while in primary was absent. The highest IgE values were detected in samples collected after deferves-

cence. IgE levels in sera were lower respect to IgM and IgA.

Isotype switching to IgE requires 2 signals: one signal is provided by IL-4 or IL-13, which activates transcription at the IgE isotype-specific; the second that is provided by ligation of CD40 on B cells by CD40L on T cells, which activates DNA switch recombination [49]. The activation of the specific T helper 2 (Th2) cells leads to expression of IL-4 and IL-13, and induction of class switching to IgE. Although T cells are the source of both activation signals, basophile cells express high levels of IL-4 and IL-13 after activation and have been suggested to play a role in both polyclonal amplification of IgE production and differentiation of Th2 cells, very similar to human mast cells which produce IL-4 in minor amount than basophiles but could play a role in secondary dengue infection [49] [50]. In dengue infections some pathologies has been associated with the development of the several clinical picture, such as diabetes, sickle cell anaemia and bronchial asthma [51] [52]. This last has been observed in patients with a higher production of IgE and predisposition to a Th2 cell response, constituting a risk for the severe illness [43], although we cannot assure that it was the cause of some patients in this work. The use of IgE as a possible prognostic marker of severe illness and the role of this antibody in the pathogenesis of dengue infection should be more studied.

## 5. Conclusion

This paper shows the acute phase of illness as the best period for the detection of dengue NS1 protein in human sera and suggests its utility as early and effective diagnostic marker. IgM detection is still an invaluable tool for routine dengue diagnosis but for samples collected after day 5 of onset of fever. Therefore, it's considered a delay diagnostic, representing a difficulty for definition of recent infection case, mainly in countries where this illness is endemic. IgA response could define a secondary infection in early acute phase of illness (between days 0 and 4), demonstrating its utility in (hyper)-endemic areas where more cases are frequently associated with severe outcomes. Finally, IgA and IgE could be evaluated as prognostic marker associated to several picture of illness in secondary dengue infections.

## Funding

This research was supported by the European Commission grant No. 517708, 6th Framework Programme. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Competing Interests

The authors have declared that no competing interests exist.

## Ethical Approval

Ethical approval was obtained from the Ethics Review Committee of WHO and each institution involved in DENCO project (A multi-centre prospective obser-

ational study of dengue in Southeast Asia and the Americas).

## References

- [1] Yacoub, S., Mongkolsapaya, J. and Screaton, G. (2016) Recent Advances in Understanding Dengue. *F1000Research*, **5**, F1000.
- [2] Guzman, M.G., Halstead, S.B., Artsob, H., Buchy, P., Farrar, J., Gubler, D.J., Hunsperger, E., Kroeger, A., Margolis, H.S., Martinez, E., Nathan, M.B., Pelegrino, J.L., Simmons, C., Yoksan, S. and Peeling, R.W. (2010) Dengue: A Continuing Global Threat. *Nature Reviews Microbiology*, **8**, S7-S16.  
<https://doi.org/10.1038/nrmicro2460>
- [3] Halstead, S.B. and Cohen, S.N. (2015) Dengue Hemorrhagic Fever at 60 Years: Early Evolution of Concepts of Causation and Treatment. *Microbiology and Molecular Biology Reviews*, **79**, 281-291. <https://doi.org/10.1128/MMBR.00009-15>
- [4] Guzman, M.G. and Kouri, G. (1996) Advances in Dengue Diagnosis. *Clinical and Diagnostic Laboratory Immunology*, **3**, 621-627.
- [5] WHO (1997) Dengue Hemorrhagic Fever. Diagnosis, Treatment, Prevention and Control. Geneva, 1-84.
- [6] Groen, J., Koraka, P., Velzing, J., Copra, C. and Osterhaus, A.D. (2000) Evaluation of Six Immunoassays for Detection of Dengue Virus-Specific Immunoglobulin M and G Antibodies. *Clinical and Diagnostic Laboratory Immunology*, **7**, 867-871.
- [7] Muller, D.A. and Young, P.R. (2013) The Flavivirus NS1 Protein: Molecular and Structural Biology, Immunology, Role in Pathogenesis and Application as a Diagnostic Biomarker. *Antiviral Research*, **98**, 192-208.
- [8] Tricou, V., Vu, H.T., Quynh, N.V., Nguyen, C.V., Tran, H.T., Farrar, J., Wills, B. and Simmons, C.P. (2010) Comparison of Two Dengue NS1 Rapid Tests for Sensitivity, Specificity and Relationship to Viraemia and Antibody Responses. *BMC Infectious Diseases*, **10**, 142. <https://doi.org/10.1186/1471-2334-10-142>
- [9] De Decker, S., Vray, M., Sistek, V., Labeau, B., Enfissi, A., Rousset, D. and Matheus, S. (2015) Evaluation of the Diagnostic Accuracy of a New Dengue IgA Capture Assay (Platelia Dengue IgA Capture, Bio-Rad) for Dengue Infection Detection. *PLoS Neglected Tropical Diseases*, **9**, e0003596.  
<https://doi.org/10.1371/journal.pntd.0003596>
- [10] Koraka, P., Suharti, C., Setiati, T.E., Mairuhu, A.T., Van Gorp, E., Hack, C.E., Jufrie, M., Sutaryo, J., Van Der Meer, G.M., Groen, J. and Osterhaus, A.D. (2001) Kinetics of Dengue Virus-Specific Serum Immunoglobulin Classes and Subclasses Correlate with Clinical Outcome Of Infection. *Journal of Clinical Microbiology*, **39**, 4332-4338. <https://doi.org/10.1128/JCM.39.12.4332-4338.2001>
- [11] Guzman, M.G., Jaenisch, T., Gaczowski, R., Ty Hang, V.T., Sekaran, S.D., Kroeger, A., Vazquez, S., Ruiz, D., Martinez, E., Mercado, J.C., Balmaseda, A., Harris, E., Dimano, E., Leano, P.S., Yoksan, S., Villegas, E., Benduzu, H., Villalobos, I., Farrar, J. and Simmons, C.P. (2010) Multi-Country Evaluation of the Sensitivity and Specificity of Two Commercially-Available NS1 ELISA Assays for Dengue Diagnosis. *PLoS Neglected Tropical Diseases*, **4**, e811.
- [12] Lanciotti, R.S., Calisher, C.H., Gubler, D.J., Chang, G.J. and Vorndam, A.V. (1992) Rapid Detection and Typing of Dengue Viruses from Clinical Samples by Using Reverse Transcriptase-Polymerase Chain Reaction. *Journal of Clinical Microbiology*, **30**, 545-551.
- [13] Vazquez, S., Perez, A.B., Ruiz, D., Rodriguez, R., Pupo, M., Calzada, N., González, L., González, D., Castro, O., Serrano, T. and Guzmán, M.G. (2005) Serological

- Markers during Dengue 3 Primary and Secondary Infections. *Journal of Clinical Virology*, **33**, 132-137. <https://doi.org/10.1016/j.jcv.2004.10.013>
- [14] Vazquez, S., Acosta, N., Ruiz, D., Calzada, N., Alvarez, A.M. and Guzman, M.G. (2009) Immunoglobulin G Antibody Response in Children and Adults with Acute Dengue 3 Infection. *Journal of Virological Methods*, **159**, 6-9.
- [15] WHO (2013) Dengue and Severe Dengue. Media Centre, Updated September. Fact Sheet N 117.
- [16] WHO/TDR (2009) Dengue Case Classification. In: *Dengue, Guidelines for Diagnosis Treatment, Prevention and Control*, Geneva, 1-146.
- [17] Clark, D.H. and Casals, J. (1958) Techniques for Hem-Agglutination-Inhibition with Arthropod-Borne Viruses. *American Journal of Tropical Medicine and Hygiene*, **7**, 561-573.
- [18] Gonzalez, D., Castro, O., Perez, J., Martinez, E., Vazquez, S., Rosario, D., Cancio, R. and Guzman, M.G. (2005) Classical Dengue Hemorrhagic Fever Resulting from Two Dengue Infections Spaced 20 Years or More Apart: Havana, Dengue 3 Epidemic, 2001-2002. *International Journal of Infectious Diseases*, **9**, 280-285. <https://doi.org/10.1016/j.ijid.2004.07.012>
- [19] Gutsche, I., Coulibaly, F., Voss, J.E., Salmon, J., d'Alayer, J., Ermonval, M., Larquet, E., Charneau, P., Krey, T., Megret, F., Guittet, E., Rey, F.A. and Flamand, M. (2011) Secreted Dengue Virus Nonstructural Protein NS1 Is an Atypical Barrel-Shaped High-Density Lipoprotein. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 8003-8008. <https://doi.org/10.1073/pnas.1017338108>
- [20] Shan, X., Wang, X., Yuan, Q., Zheng, Y., Zhang, H., Wu, Y. and Yang, J. (2015) Evaluation of the Diagnostic Accuracy of Nonstructural Protein 1 Ag-Based Tests for Dengue Virus in Asian Population: A Meta-Analysis. *BMC Infectious Diseases*, **15**, 360. <https://doi.org/10.1186/s12879-015-1088-4>
- [21] Zhang, H., Li, W., Wang, J., Peng, H., Che, X., Chen, X. and Zhou, Y. (2014) NS1-Based Tests with Diagnostic Utility for Confirming Dengue Infection: A Meta-Analysis. *International Journal of Infectious Diseases*, **26**, 57-66. <https://doi.org/10.1016/j.ijid.2014.02.002>
- [22] Hang, V.T., Nguyet, N.M., Trung, D.T., Tricou, V., Yoksan, S., Dung, N.M., Van Ngoc, T., Hien, T.T., Farrar, J., Wills, B. and Simmons, C.P. (2009) Diagnostic Accuracy of NS1 ELISA and Lateral Flow Rapid Tests for Dengue Sensitivity, Specificity and Relationship to Viraemia and Antibody Responses. *PLoS Neglected Tropical Diseases*, **3**, e360. <https://doi.org/10.1371/journal.pntd.0000360>
- [23] Tricou, V., Minh, N.N., Farrar, J., Tran, H.T. and Simmons, C.P. (2011) Kinetics of Viremia and NS1 Antigenemia Are Shaped by Immune Status and Virus Serotype in Adults with Dengue. *PLoS Neglected Tropical Diseases*, **5**, e1309. <https://doi.org/10.1371/journal.pntd.0001309>
- [24] Alcon, S., Talarmin, A., Debruyne, M., Falconar, A., Deubel, V. and Flamand, M. (2002) Enzyme-Linked Immunosorbent Assay Specific to Dengue Virus Type 1 Nonstructural Protein NS1 Reveals Circulation of the Antigen in the Blood during the Acute Phase of Disease in Patients Experiencing Primary or Secondary Infections. *Journal of Clinical Microbiology*, **40**, 376-381. <https://doi.org/10.1128/JCM.40.02.376-381.2002>
- [25] Shu, P.Y., Chen, L.K., Chang, S.F., Yueh, Y.Y., Chow, L., Chien, L.J., Chin, C., Lin, T.H. and Huang, J.H. (2003) Comparison of Capture Immunoglobulin M (IgM) and IgG Enzyme-Linked Immunosorbent Assay (ELISA) and Nonstructural Protein NS1 Serotype-Specific IgG ELISA for Differentiation of Primary and Secondary Dengue Virus Infections. *Clinical and Diagnostic Laboratory Immunology*, **10**, 622-

630.

- [26] Dussart, P., Labeau, B., Lagathu, G., Louis, P., Nunes, M.R., Rodrigues, S.G., Storck-Herrmann, C., Cesaire, R., Morvan, J., Flamand, M. and Baril, L. (2006) Evaluation of an Enzyme Immunoassay for Detection of Dengue Virus NS1 Antigen in Human Serum. *Clinical and Vaccine Immunology*, **13**, 1185-1189. <https://doi.org/10.1128/CVI.00229-06>
- [27] Xu, H., Di, B., Pan, Y.X., Qiu, L.W., Wang, Y.D., Hao, W., He, L.J., Yuen, K.Y. and Che, X.Y. (2006) Serotype 1-Specific Monoclonal Antibody-Based Antigen Capture Immunoassay for Detection of Circulating Nonstructural Protein NS1: Implications for Early Diagnosis and Serotyping of Dengue Virus Infections. *Journal of Clinical Microbiology*, **44**, 2872-2878. <https://doi.org/10.1128/JCM.00777-06>
- [28] Hu, D., Di, B., Ding, X., Wang, Y., Chen, Y., Pan, Y., Wen, K., Wang, M. and Che, X. (2011) Kinetics of Non-Structural Protein 1, IgM and IgG Antibodies in Dengue Type 1 Primary Infection. *Virology Journal*, **8**, 47. <https://doi.org/10.1186/1743-422X-8-47>
- [29] Guzman, M.G. and Kouri, G. (2004) Dengue Diagnosis, Advances and Challenges. *International Journal of Infectious Diseases*, **8**, 69-80. <https://doi.org/10.1016/j.ijid.2003.03.003>
- [30] Summers, P.L., Eckels, K.H., Dalrymple, J.M., Scott, R.M. and Boyd, V.A. (1984) Antibody Response to Dengue-2 Vaccine Measured by Two Different Radioimmunoassay Methods. *Journal of Clinical Microbiology*, **19**, 651-659.
- [31] Innis, B.L., Nisalak, A., Nimmannitya, S., Kusalerdchariya, S., Chongswasdi, V., Suntayakorn, S., Puttisri, P. and Hoke, C.H. (1989) An Enzyme-Linked Immunosorbent Assay to Characterize Dengue Infections Where Dengue and Japanese Encephalitis Co-Circulate. *The American Journal of Tropical Medicine and Hygiene*, **40**, 418-427.
- [32] Nogueira, R.M., Miagostovich, M.P., Cavalcanti, S.M., Marzochi, K.B. and Schatzmayr, H.G. (1992) Levels of IgM Antibodies against Dengue Virus in Rio de Janeiro, Brazil. *Research in Virology*, **143**, 423-427.
- [33] Klein, U., Goossens, T., Fischer, M., Kanzler, H., Braeuninger, A., Rajewsky, K. and Kuppers, R. (1998) Somatic Hypermutation in Normal and Transformed Human B Cells. *Immunological Reviews*, **162**, 261-280. <https://doi.org/10.1111/j.1600-065X.1998.tb01447.x>
- [34] Agematsu, K., Hokibara, S., Nagumo, H. and Komiyama, A. (2000) CD27: A Memory B-Cell Marker. *Trends in Immunology*, **21**, 204-206. [https://doi.org/10.1016/s0167-5699\(00\)01605-4](https://doi.org/10.1016/s0167-5699(00)01605-4)
- [35] Kruetzmann, S., Rosado, R., Weber, H., Germing, U., Tournilhac, O., Peter, H., Berner, R., Peters, A., Boehm, T., Plebani, A., Quinti, I. and Carsetti, R. (2003) Human Immunoglobulin M Memory B Cells Controlling *Streptococcus pneumonia* Infections Are Generated in the Spleen. *Journal of Experimental Medicine*, **197**, 939-945. <https://doi.org/10.1084/jem.20022020>
- [36] Weller, S., Braun, M.C., Tan, B.K., Rosenwald, A., Cordier, C., Conley, M.E., Plebani, A., Kumararatne, D.S., Bonnet, D., Tournilhac, O., Tchernia, G., Steiniger, B., Staudt, L.M., Casanova, J.-L., Reynaud, C.-A. and Weill, J.-C. (2004) Human Blood IgM "Memory" B Cells Are Circulating Splenic Marginal Zone B Cells Harboring a Prediversified Immunoglobulin Repertoire. *Blood*, **104**, 3647-3654. <https://doi.org/10.1182/blood-2004-01-0346>
- [37] White, H. and Gray, D. (2000) Analysis of Immunoglobulin (Ig) Isotype Diversity and IgM/D Memory in the Response to Phenyl-Oxazolone. *Journal of Experimental Medicine*, **191**, 2209-2219. <https://doi.org/10.1084/jem.191.12.2209>

- 
- [38] Shi, Y., Agematsu, K., Ochs, H. and Suganea, K. (2003) Functional Analysis of Human Memory B-Cell Subpopulations: IgD CD27 B Cells Are Crucial in Secondary Immune Response by Producing High Affinity IgM. *Clinical Immunology*, **108**, 128-137.
  - [39] Naz, A., Zahid, D., Murky, S.N., Nadeem, M., Sil, B.K. and Shamsi, T.S. (2014) Evaluation of Efficacy of Various Immunochromatographic Rapid Tests for Dengue Diagnosis. *Pakistan Journal of Medical Sciences*, **30**, 166-171.
  - [40] Balmaseda, A., Guzman, M.G., Hammond, S., Robleto, G., Flores, C., Tellez, Y., Vide, E., Saborio, S., Perez, L., Sandoval, E., Rodriguez, Y. and Harris, E. (2003) Diagnosis of Dengue Virus Infection by Detection Of Specific Immunoglobulin M (IgM) and IgA Antibodies in Serum and Saliva. *Clinical and Diagnostic Laboratory Immunology*, **10**, 317-322.
  - [41] Nawa, M., Takasaki, T., Ito, M., Inoue, S., Morita, K. and Kurane, I. (2005) Immunoglobulin A Antibody Responses in Dengue Patients: A Useful Marker for Serodiagnosis of Dengue Virus Infection. *Clinical and Diagnostic Laboratory Immunology*, **12**, 1235-1237.
  - [42] Blacksell, S.D., Jarman, R.G., Gibbons, R.V., Tanganuchitcharnchai, A., Mammen Jr., M.P., Nisalak, A., Kalayanaroj, S., Bailey, M.S., Premaratna, R., de Silva, H.J., Day, N.P. and Laloo, D.G. (2012) Comparison of Seven Commercial Antigen and Antibody Enzyme-Linked Immunosorbent Assays for Detection of Acute Dengue Infection. *Clinical and Vaccine Immunology*, **19**, 804-810.  
<https://doi.org/10.1128/CVI.05717-11>
  - [43] Pérez, A.B., García, G., Sierra, B., Alvarez, M., Vázquez, S., Cabrera, M.V., Rodriguez, R., Rosario, D., Martinez, E., Denny, T. and Guzmán, M.G. (2004) IL-10 Levels in Dengue Patients: Some Findings from the Exceptional Epidemiological Conditions in Cuba. *Journal of Medical Virology*, **73**, 230-234.  
<https://doi.org/10.1002/jmv.20080>
  - [44] Sierra, B., Perez, A.B., Vogt, K., Garcia, G., Schmolke, K., Aguirre, E., Alvarez, M., Kern, F., Kouri, G., Volk, H.D. and Guzman, M.G. (2010) Secondary Heterologous Dengue Infection Risk: Disequilibrium between Immune Regulation and Inflammation? *Cellular Immunology*, **262**, 134-140.
  - [45] Pate, M., Smith, J., Chi, D. and Krishnaswamy, G. (2010) Regulation and Dysregulation of Immunoglobulin E: A Molecular and Clinical Perspective. *Clinical and Molecular Allergy*, **8**, 3. <https://doi.org/10.1186/1476-7961-8-3>
  - [46] Alexeyev, O.A., Ahlm, C., Billheden, J., Settergren, B., Wadell, G. and Juto, P. (1994) Elevated Levels of Total and Puumala Virus-Specific Immunoglobulin E in the Scandinavian Type of Hemorrhagic Fever with Renal Syndrome. *Clinical and Diagnostic Laboratory Immunology*, **1**, 269-272.
  - [47] Miguez-Burbano, M.J., Jaramillo, C.A., Palmer, C.J., Shor-Posner, G., Velasquez, L.S., Lai, H. and Baum, M.K. (1999) Total Immunoglobulin E Levels and Dengue Infection on San Andres Island, Colombia. *Clinical and Diagnostic Laboratory Immunology*, **6**, 624-626.
  - [48] Koraka, P., Murgue, B., Deparis, X., Setiati, T.E., Suharti, C., van Gorp, E.C., Hack, C.E., Osterhaus, A.D. and Groen, J. (2003) Elevated Levels of Total and Dengue Virus-Specific Immunoglobulin E in Patients with Varying Disease Severity. *Journal of Medical Virology*, **70**, 91-98. <https://doi.org/10.1002/jmv.10358>
  - [49] Stone, K., Prussin, C. and Metcalfe, D. (2010) IgE, Mast Cells, Basophils, and Eosinophils. *Journal of Allergy and Clinical Immunology*, **125**, S73-S80.  
<https://doi.org/10.1016/j.jaci.2009.11.017>
  - [50] Vercelli, D. (2009) Immunobiology of IgE. In: Adkinson, N., Bochner, B.S., Busse,



W.W., Holgate, S.T., Lemanske, R.F. and Simons, F.E.R., Eds., *Middleton's Allergy: Principles and Practice*, 7th Edition, Mosby Elsevier, St Louis.

- [51] Kouri, G.P., Guzman, M.G. and Bravo, J.R. (1987) Why Dengue Haemorrhagic Fever in Cuba? 2. An Integral Analysis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81**, 821-823.  
[https://doi.org/10.1016/0035-9203\(87\)90042-3](https://doi.org/10.1016/0035-9203(87)90042-3)
- [52] Guzman, M.G. and Kouri, G. (2008) Dengue Haemorrhagic Fever Integral Hypothesis: Confirming Observations, 1987-2007. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **102**, 522-523.  
<https://doi.org/10.1016/j.trstmh.2008.03.001>



Open Access Library

**Submit or recommend next manuscript to OALib Journal and we will provide best service for you:**

- Publication frequency: Monthly
- 9 [subject areas](#) of science, technology and medicine
- Fair and rigorous peer-review system
- Fast publication process
- Article promotion in various social networking sites (LinkedIn, Facebook, Twitter, etc.)
- Maximum dissemination of your research work

Submit Your Paper Online: [Click Here to Submit](#)

Or Contact [service@oalib.com](mailto:service@oalib.com)