International Journal of Infectious Diseases 100 (2020) 316–323

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Predominant secondary dengue infection among Vietnamese adults mostly without warning signs and severe disease

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ARTICLE INFO

Article history:
Received 14 May 2020
Received in revised form 27 August 2020
Accepted 30 August 2020

Keywords:
Dengue
DENV serotypes
IgM:IgG ratio
Primary infection
Secondary infection
Thrombocytopenia
Hospital stay

ABSTRACT

Background: The morbidity in dengue fever is dependent on the dengue virus (DENV) serotypes, the patient age, predisposing immunogenic markers and the frequency of primary and secondary infections. This study aims to distinguish acute primary from secondary dengue infections of Vietnamese adults and to assess the association of viremia and anti-dengue immunoglobulin levels with clinical outcomes.
Study design: Viral RNA, dengue serotypes and levels of anti-dengue IgM and IgG of hospitalized adult cases were determined in EDTA-plasma samples prospectively collected during three consecutive years of dengue infection in Hanoi. Patients admitted to hospital within 7 days of their 1st reported fever were included. Primary infections were anti-dengue IgG enzyme-linked immunosorbent assay (ELISA) negative on both day of hospital entry (day 0) and day two or three of hospitalization (day 2 or 3) with a positive anti-dengue IgM on either day 0 or day 2 or 3 hospitalization. The secondary infections were anti-dengue IgG ELISA positive on both day 0 and day 2 or 3 with positive anti-dengue IgM ELISA on either day 0 or day 2 or 3.
Results: The hospitalized dengue fever cases between October 2016 and March 2019 were predominantly secondary infections (74%, 68% and 77%, respectively) with DENV-1 (60% and 65%) and DENV-2 (22% and 26%) serotypes determined in the latter two years. The viremia in primary infection was significantly higher than that in secondary infection ($P < 0.01$) and positively correlated with the days of hospital stay. In secondary infections, platelet counts were lower than in primary infections ($P = 0.04$) and IgG levels in secondary infection negatively correlated with platelet counts (Spearman's $r = -0.22$, $P < 0.01$).
Conclusions: Our results indicate high rates of secondary infection with DENV1 and DENV2 serotypes. Anti-dengue immunoglobulins negatively correlate with hospital stay and platelet counts with few warning signs or severe disease. Further investigations of specific antibodies in adults which predict auto-inflammatory activity after the recovery from dengue infection are warranted.

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https://doi.org/10.1016/j.ijid.2020.08.082

Introduction

Dengue is a febrile infectious disease caused by the single stranded (ss) +RNA dengue virus (DENV) which is transmitted by mosquitos in four DENV serotypes (DENV1–4) of which DENV1 and DENV2 are predominant in Vietnam (Aguas et al., 2019; Lam et al., 2017). The critical phase of a patient infection, 3–7 days after the onset of fever, is usually self-limited but inflammatory cytokine and chemokine responses together with persistent viremia may induce haemorrhages and liver damage in severe dengue haemorrhagic fever (DHF) or life-threatening respiratory and cardiovascular distress in dengue shock syndrome (DSS) (Hung et al., 2019; St John and Rathore, 2019; Vaughn et al., 2000). An estimated 390 million new dengue infections occur in tropical and subtropical countries each year and about 25–30 percent are clinical cases involving hospitalization (Bhatt et al., 2013; Hung et al., 2018). The high urban population density, humidity and abundant mosquito breeding grounds perpetuate seasonal outbreaks of dengue with morbidity largely dependent on the infecting virus serotypes and the frequency of primary versus secondary and further subsequent infections (World Health Organization, 2020).

Antibody responses typically serve as an adaptive protective barrier against viral infections. Dengue-specific immunoglobulins which bind the viral envelope or surface proteins and block the host membrane receptor-mediated uptake of viral particles into host cells are referred to as neutralizing antibodies. Although a primary infection with one DENV serotype provides long term homologous protective immunity, a subsequent infection with a different DENV serotype is associated with worsening clinical outcome (St John and Rathore, 2019). The severity of secondary infection has been attributed to antibody-dependent enhancement (ADE), a mechanism whereby the antibodies lacking neutralizing activity and produced during primary infection, cross-react with new DENV subtype in secondary infection to enhance the virus entry into target cells bearing Fc-gamma (FcRγ) or complement receptors thus increasing viral persistence in macrophages and worsening the clinical symptoms (Guzman et al., 2013; Guzman and Kouri, 2004; Vaughn et al., 2000).

During primary infections, IgM antibodies are the first immunoglobulin isotype to appear (De Paula and Fonseca, 2004). Few patients have detectable anti-dengue IgM antibodies by the 2nd to the 4th day after the beginning of the symptoms, 80% of all dengue cases show anti-dengue IgM antibodies by day 5 of illness, and 93–99% have detectable IgM by day 6 to day 10 post-onset of symptoms, which may then remain for over 90 days (Guzman et al., 2013). Low levels of anti-dengue IgGs are detectable at the end of the first week of illness and are still visible after several months and may even persist for life (St John and Rathore, 2019). During secondary infections, anti-DENV IgG antibodies appear either before or along with IgM antibodies. Furthermore, IgM antibody titers tend to be significantly lower in secondary than primary infections. The non-structural protein 1 (NS1), secreted early in dengue infection and vital for viral assembly, contributes to the pathogenesis (Glasner et al., 2018).

The age-specific severity of primary and secondary dengue has been documented in southern Vietnam where severe dengue and DHF/DSS were reported more frequently among infants and children than in adults (Thai et al., 2011). Although severe dengue with vascular leakage and DSS is more common among children than adults (Trung et al., 2012; Rosenberger et al., 2020), loss of platelets and bleeding is greater in adults than children who have no dengue complications and do not go on to develop DSS (Trung et al., 2012). The economic burden of Dengue in Vietnam, estimated at 95 million USD for the year 2016 is exacerbated by an ever-increasing dengue infection rate; 38,000 reported in 2019 representing a 3.6% increase compared to 2018 (World Health Organization, 2020). However, health insurance reimbursements were insufficient to cover hospitalization costs (McBride et al., 2019).

The management of adult acute dengue infection in endemic regions, having high rates of secondary infection, particularly during outbreaks is of increasing importance for the risk of Dengue in the blood supply. To prevent viraemic blood transfusions, donors with Dengue in endemic regions are restricted for 6 months or more after infection and subjected to screening tests for primary and secondary infection. Educational programs in northern Vietnam have vastly improved the knowledge, attitudes and practices of the general population (Nguyen et al., 2019).

Previous studies have validated a classification method to discriminate between primary and secondary dengue infections based on software algorithm of ELISA IgG antibody levels and the number of days of symptoms (Cordeiro et al., 2009). Other studies use titers of the plaque reduction neutralization assay (Nguyen et al., 2018) or ELISA (Changal et al., 2016) to calculate the ratio of IgM:IgG at hospitalization and to determine the primary and secondary infections. The most reliable way to define primary and secondary dengue infection is based on a combination of multiple laboratory tests that detect dengue viral RNA and dengue-specific antibodies (Cordeiro et al., 2009; Nisalak, 2015; Nguyen et al., 2019). The clinical laboratories in dengue-endemic countries cannot realistically perform both serological and molecular assays on all samples (Kikuti et al., 2019; Nguyen et al., 2018).

The NS1 and IgM/IgG duo lateral flow rapid test is a cost-effective and labour-saving point-of-care test which facilitates quick decision making on the allocation of hospital resources and beds during dengue outbreaks. The intended use of the NS1 component of the duo rapid test is to aide in the diagnosis of early febrile dengue (Kikuti et al., 2019). The rapid testing is not quantitative and therefore not reliable to differentiate primary and secondary Dengue infection (Rockstroh et al., 2019). Proper definition and accurate assignment of the primary versus secondary status is notoriously difficult since there is no gold standard (Nguyen et al., 2018).

For this reason, we distinguish primary and secondary infection in adult dengue patients of North Vietnam over three consecutive years by the anti-dengue IgM and anti-dengue IgG ELISA. Furthermore the viremia and DENV serotypes in primary and secondary infection are determined using molecular tests. The levels of dengue-specific immunoglobulins in primary versus secondary infection were measured at the day of hospital entry and at two follow up time points during the hospitalization. The hospital stay and the duration of illness, defined by the number of days after the estimated day of first reported fever, were assessed for their associations with viremia. The associations between levels of dengue-specific immunoglobulins and thrombocytopenia or dengue classification were evaluated in primary and secondary infections.

Materials and methods

Ethics statement

Written informed consent was obtained from all study participants prior to recruitment. The study was approved by the Institutional Review Board of Vietnam Military Medical University (VMMU), Hanoi, Vietnam (Nr. 103MCH/RES/DENV-GER_V-D1-2016) and the 108 Hospital (Nr. Nr.108MCH/RES/DENV_D1-08-05-2018-SDL).

Study subjects and sampling procedures

Dengue patients were recruited for three consecutive years in accordance with 2009 WHO guidelines (WHO, 2009), ethics committee review board approved the study plan and informed written patient consent. All patients on the day of admission had reported their onset of first fever within 7 days and were screened for NS1 antigen, dengue-specific IgM and dengue-specific IgG.

The assignment of primary infections was according to IgG negative result by ELISA (values <10 U/L) at both day 0 and day 2 or 3 of hospitalization with IgM ELISA positive (≥10 U/L) on either day 0 or day 2 or 3 of hospitalization. The secondary infections were assigned according to IgG positive by ELISA (≥10 U/L) at both day 0 and day 2 or 3 hospitalization with IgM positive ELISA (≥10 U/L) on either day 0 or day 2 or 3 hospitalization.

The first cohort of febrile patients was hospitalized at the 103 Military Hospital of VMMU, Hanoi, Vietnam during seasonal outbreak (Nov 2016–Feb 2017); seventy-two enrolled, 38 males, 34 females; mean age ± SD: 35 ± 15 years. This cohort was evaluated on hospital admission and on day 2 or day 3. The second cohort of Nov 2017–Feb 2018 outbreak with 80 enrolled (46 males, 42 females), mean age ± SD; 36 ± 13 years, and the third cohort of 65 febrile patients (48 males, 33 females) at the 108 Military Hospital during the Oct 2018-Apr 2019 outbreak, mean age ± SD; 41 ± 15 years, were evaluated at day 0 hospital admission, on day 2 or 3, and on day 7–10 days hospitalization. The hospitalized cases were from Hanoi districts and surrounding areas. The EDTA plasma of DENV patients was stored at −80 °C, until further use. The baseline characteristics of all three cohorts are summarized in Table 1. The warning signs included abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation (pleural effusion or ascites), mucosal bleed, lethargy or drowsiness, hepatomegaly, and an increase in hematocrit level along with a rapid decrease in platelet count. Severe dengue was defined as plasma leakage (hematocrit change >20%) with narrow pulse <20 mm Hg mmHg), fluid accumulation with respiratory distress, or severe bleeding or organ impairment. Based on NS1 or IgM positivity and/or severity of their signs and symptoms, patients were given intravenous hydration and supportive care treatment.

Detection of DENV-RNA and DENV-specific antibodies

The viral RNA was extracted from 130 μL patient plasma by Cobas® Magnumpure (Roche, Penzberg, Germany) for cohort 1 or manual RNA isolation (viral RNAeasy Qiagen Hilden, Germany) for cohorts 2 and 3. The detection and quantification of viral RNA was by Cobas® AmpliprepCobas® TaqMan, cohort 1, or ModularDx Dengue 58-0700-96 with EAV extraction control 66-0909-96 LightMix on Roche LightCycler 480, cohorts 2 and 3. DENV1-4 serotyping was performed using the Dengue Typing kit 40-0700-2 (TIB Molbiol Berlin, Germany) on Roche LightCycler 480. DENV specific antibodies were determined by enzyme-linked immunosorbent assay (ELISA) of SERION ELISA Dengue Virus classic IgM and IgG (Serion GmbH, Würzburg, Germany) following the manufacturer's instructions. The measuring ranges of quantification for the IgM test and for the IgG test were 5–200 U/mL and 5–600 U/mL, respectively with cut-off range of 10–15 U/L.

Statistical analysis

The IgM and IgG levels are presented as mean with standard deviation and medians with ranges. Comparisons of the values between patient groups were assessed by non-parametric Mann–Whitney sum rank test. A *p* value of 0.05 was considered to be statistically significant with differences between independent groups. Correlation analyses were calculated with the Spearman's rank correlation coefficients. The correlation coefficients of $r > 0.4$ or $r < -0.4$ with significance at $p < 0.05$ were considered strong positive or strong negative associations, respectively. Statistical analysis was done with MedCalc version 14 for Windows (MedCalc Software, Mariakerke, Belgium).

Results

Patient characteristics and infection status

The median age and gender ratios of the dengue patients were not significantly different between the three cohorts (Table 1). The patients belonging to cohort 2 and cohort 3 had median hospital stays of 5 days and 6 days respectively. The patients in primary infection cohort 2 and cohort 3 were hospitalized within median 2 or 3 days of their first reported fever respectively, significantly earlier than the patients in secondary infection; 5 or 4 days respectively (Table 1). Thrombocytopenia and hepatic inflammation were characteristic of the cases in all three dengue cohorts indicated by the median platelet counts below the normal range and the median liver enzyme activity above the normal range, respectively (Table 1).

Fever, headache, body ache and fatigue were the most common signs and symptoms on hospital admission. Dengue with warning signs for the three cohorts shows a trend of higher percentage among secondary infection (4%, 40% and 21%) than among primary infection (0%, 33% and 17%) but the differences in percent of patients with warning signs did not reach significance (Table 1). Vomiting, bleeding, rash and organ impairment were the most frequent warning signs reported in the cohort 2. On the day 0 hospital admission mean IgM:IgG ratios of primary infections were ≥2.9 and the mean IgM:IgG ratios of secondary infections were ≤1.8 for all three cohorts (Table 1). All three cohorts consistently show a high rate of secondary infections; 77%, 62% and 72% respectively with a higher percent DENV-RNA positivity among primary infections; 88%, 97% and 94% respectively, than in the secondary infections; 77%, 84% and 79% respectively. The predominant serotypes of cohort 2 and cohort 3 were DENV-1 and DENV-2. The percentage of patients infected with mixed DENV-1/2 (11%) and DENV-4 (4%) serotypes in cohort 3 were higher than in cohort 2, DENV-1/2 (1%) and DENV-4 (0%). DENV-3 serotype was not detected (Table 1).

Anti-DENV IgM and IgG profile in primary versus secondary infection

To compare the profile of IgM and IgG responses in dengue primary versus secondary infection, the distributions of IgM:IgG ratios on day 0 of hospital admission and the two time intervals after hospitalization; day 2 or 3 and days 7–10 hospitalization are plotted (Figure 1). The difference between mean IgM:IgG ratio of primary dengue infections versus mean IgM:IgG ratio of secondary dengue infections were of high significance at each of the three hospitalization time points (Figure 1). The IgM:IgG cut-off <0.7 for secondary infection was calculated by receiver to operator curve (ROC) analyses over two intervals of dengue illness, days after first reported fever, from the pooled data sets of cohort 2 and cohort 3 (Figure 1). In primary infections the IgM:gG ratios ≥0.7 cut-off were 39 of 41 (95%) on day 0, 41 of 41 (100%) on day 2 or 3 hospitalization and 16 of 21 (76%) on day 7–10 hospitalization. In secondary infections the IgM:IgG ratios <0.7 cut-off were 54 of 87 (62%) on day 0, 55 of 81 (68%) on day 2 or 3 hospitalization and 33 of 52 (64%) on day 7–10 hospitalization (Figure 1).

Dengue viral RNA levels and DENV serotypes in primary versus secondary infection

The Ct values for the combined data of cohort 2 and cohort 3 were significantly lower in primary infection compared to secondary infection indicating higher plasma viral RNA concentrations in the primary infection than secondary infection. The rank order of Ct

Table 1
Patient characteristics of seasonal Dengue outbreaks in Hanoi.

Characteristics	2016–2017 Cohort 1	2017–2018 Cohort 2	2018–2019 Cohort 3	P value (1)	P value (2)	P value (3)
Number of cases enrolled	N = 69	N = 80	N = 65			
Age Median [Range] years	30 [20–63]	32 [20–80]	39 [17–72]	NS	NS	NS
Gender M/F	37/32	43/37	36/26	NS	NS	NS
Median Hospital Stay [Range]	NA	5 [2–11]	6 [3–19]	–	–	NS
AST Mean U/L (95%CI)	79 (62–97)	76 (36–116)	74 (61–88)	NS	NS	NS
ALT Mean U/L (95%CI)	61 (48–74)	53 (29–77)	52 (42–61)	NS	NS	NS
Leucocytes/μL mean (95%CI)	4.7 (4–5.3)	3.6 (3.1–4.1)	4.5 (3.8–5.4)	NS	NS	NS
Lymphocyte percentage (95%CI)	NA	32 (29–35)	41 (25–56)	–	–	NS
Platelet count × 10^3 mean/μL (95%CI)	107 (92–121)	96 (86–107)	111 (91–131)	NS	NS	NS
Warning Signs n (%)						
Bleeding	NA	29 (36)	5 (7)	–	–	–
Vomittng	NA	15 (19)	1 (1)	–	–	–
Rash	NA	21(26)	9 (14)	–	–	–
Liver enlargement	NA	2 (3)	0 (0)	–	–	–
Serotype						
DENV1 n(%)	NA	51(64)	31(48)	–	–	0.15
DENV2 n(%)	NA	19(24)	13(20)	–	–	0.79
DENV1/2 n(%)	NA	1 (1)	7(11)	–	–	–
DENV3 n(%)	NA	0(0)	0(0)	–	–	–
DENV4 n(%)	NA	0(0)	3 (4)	–	–	–
Negative n (%)	17 (24)	9 (11)	11 (17)	0.43	0.66	0.7
				P value (primary vs secondary)		
Primary infection n(%)	16 (23)	30 (38)	18 (28)	**0.001**	**0.04**	**0.001**
Secondary infection n(%)	53 (77)	50 (62)	47 (72)			
*Days 1st Fever Median [range]						
Primary	≤6	2 [1–5]	3 [1–6]	NA	**0.001**	**0.004**
Secondary		5 [1–7]	4 [1–9]			
IgM/IgG ratio						
Primary mean (95%CI)	3.3 (1.0–5.5)	2.9 (1.5–4.3)	6.9 (0.34–13)	**0.001**	**0.002**	0.06
Secondary mean (95%CI)	1.3 (0.8–1.8)	1 (0.56–1.49)	1.8 (0.6–3.1)			
NAT + %						
Primary	88	97	94	0.91	0.08	0.15
Secondary	77	84	79			
NS1 Rapid Test + %						
Primary	63	97	94	**0.04**	0.2	0.69
Secondary	85	89	91			
Severe dengue n (%)						
Primary	0 (0)	0 (0)	0 (0)	0.99	0.44	0.54
Secondary	0 (0)	1 (2)	1 (2)			
Dengue + warning signs n (%)						
Primary	0 (0)	10 (33)	3 (17)	0.66	0.53	0.7
Secondary	2 (4)	20 (40)	10 (21)			
Dengue no warning signs n (%)						
Primary	16 (100)	20 (67)	15 (83)	0.88	0.53	0.6
Secondary	51 (96)	30 (60)	36 (77)			

Normal ranges; AST < 40 U/L, ALT < 40 U/L, Platelets 150–300 × 10^3/μL, Lymphocytes 1–4.8/μL, Leucocytes 4–11/μL. NA = not available. NS = not significant. (1) comparison between 2016–2017 versus 2017–2018 cohorts; (2) between 2016–2017 versus 2018–2019 cohorts; (3) between 2017–2018 versus 2018–2019 cohorts; * comparison between primary versus secondary infections. *number of days since the first fever is based on the patient reply to questioning on day of hospital admission. In cohort 1, the patients were queried for fever within 6 days.
Values $p < 0.05$ are indicated by bold.

values DENV-1 < DENV-2 < DENV-1/2 mixed serotype is the same in both the primary and secondary infections (Figure 2). The DENV-4 serotype was detected in 3 cases (Table 1) all of which belonged to the secondary infections of cohort 3 (Table 1, Figure 2) and were reported without dengue warning signs (results not shown).

Viremia and immunoglobulin levels associate with hospital stay in both primary and secondary dengue infection

The hospital stay was positively correlated with viremia (Figure 3A, C) and the days of illness negatively correlated with viremia (Figure 3B, D). To determine if the immunoglobulin levels are associated with hospital stay, the IgM and IgG levels at the second time point on day 2 or 3 hospitalization were evaluated. The IgM in primary infection (Figure 4A) and the IgG in secondary infection (Figure 4B) negatively correlated with hospital stay; the coefficient of correlation in primary infection but not in the secondary infection was of significance. The days of hospital stay, determined from the patient records on date of hospital admission to the date of hospital dismissal, was significantly longer in primary infection than in secondary infection (Figure 4C).

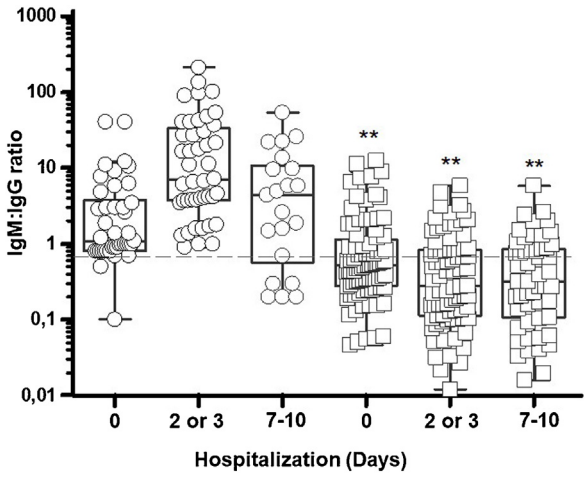

Figure 1. Time course of immunoglobulin levels in primary versus secondary dengue infections. The distribution of the ELISA IgM:IgG ratios from combined data of cohorts 2 and 3 in primary (open circles) and secondary (open squares) dengue infections are presented by box-whisker plots at three time intervals; the day of hospital entry, day 2 or day3 and day 7 to day 10 hospitalization. Horizontal dotted line IgM:IgG ratio <0.7 represents the cut-off for secondary infection calculated from receiver operator curves (ROC) analyses on days 1–4 illness; sensitivity 60% (95% CI 045–74%) and specificity 88% (95% CI 78–94% likelihood ratio 4.87 and days 5–9 illness; sensitivity 67% (95% CI 57–75%) and specificity 91% (95% CI 78–97%), likelihood ratio 7.17. **$p < 0.0001$, the difference between median IgM:IgG ratios of primary (1°) versus secondary (2°) infection is of statistical significance in the Mann–Whitney rank test.

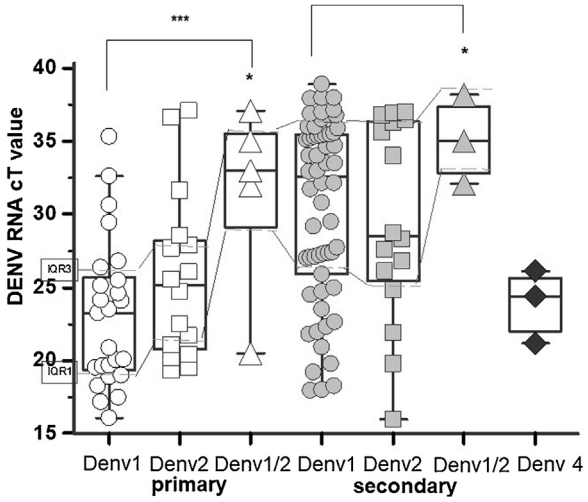

Figure 2. DENV serotypes and RNA concentration in primary and secondary infections. Distribution of the cycle threshold (Ct) values from semi-quantitative modular multiplex RT-PCR of viral RNA at day of hospital entry from combined data of cohorts 2 and 3. The DENV-RNA levels represented by Ct values of primary (open symbols) and secondary infections (closed symbols) respectively; DENV-1 (circles); DENV-2 (squares); mixed DENV-1/2 (triangles) and DENV-4 (diamond) serotypes. Mean Ct values are indicated by the horizontal line inside boxes and the Ct values within 95% CI are inside the box. The inter-quartile range (IQR) is defined by the horizontal dotted lines of lower 25 percentile (IQR-1) and upper 75 percentile (IQR-3) and compared between the serotypes by connecting lines. The differences between median Ct of primary versus secondary infection excluding DENV4 ***$p < 0.0001$ and between median Ct of DENV1 versus median Ct of DENV1/2 serotypes *$p < 0.05$ were of significance in the Mann–Whitney rank test.

Secondary IgG and platelet counts

The median platelet counts in secondary infections were significantly lower than the median platelet counts in primary infection (Figure 5A). The IgG levels in secondary infection negatively correlated with the platelet counts ($p < 0.01$) (Figure 5B). The distribution of IgG levels and the distribution of platelet counts in secondary dengue infection without warning signs and dengue with warning signs show no significant differences (Figure 5C).

Discussion

This comprehensive prospective assessment of dengue outbreaks among Northern Vietnamese adults reveals a high rate of secondary infection, predominantly of DENV1 and DENV2 serotypes with plasma viral RNA levels on the day of hospital admission significantly higher in the primary infection than in the secondary infection. The viremia, irrespective of infection status (primary vs secondary) positively correlated with hospital stay. The anti-DENV IgM in primary infection and the anti-DENV IgG in secondary infection both associate with short hospital stay and the anti-dengue IgG in secondary infection negatively correlates with platelet counts. However, the levels of anti-dengue IgG in secondary infection are equally distributed among dengue without warning signs, dengue with warning signs and severe dengue. These results suggest that the levels of anti-dengue immunoglobulins per se do not necessarily lead to worsening clinical outcomes during the course of acute adult dengue infection.

We acknowledge that only two severe cases among the three outbreaks and the absence of dengue hemorrhagic shock or mortality are not representative of adult infection in areas of South Vietnam (McBride et al., 2019) or of clinical outcomes in other dengue endemic regions of Asia which report prolonged hospital stay with mortality among severe adult dengue secondary infection (Khalil et al., 2014; Mallhi et al., 2017). Previous models and meta-analyses of dengue severity suggest that the classification of dengue and warning signs in addition to 2009 WHO guidelines can be applied to improve the accuracy for differentiating dengue severity in different dengue-endemic countries and regions of Asia due to the differences in population clinical manifestations and exposure to virulent strains of the virus (Rosenberger et al., 2020). The hospitalized dengue cases in Hanoi between October and December were later in the year than the expected peak cases between July and October in North Vietnam. This delay in timing from the typical seasonal dengue may reflect a trend of increasing temperatures and humidity reported in Hanoi Autumn and winter months (Toan et al., 2014; Cheng et al., 2020). The adult dengue cases in Hanoi with warning signs, primary infection 17–33 percent and secondary infection 21–40 percent, are consistent with the 2017 Vietnamese dengue outbreak which found dengue with warning signs among the Northern Vietnamese adults, 25 percent, lower than the Southern Vietnamese adults, 41 percent (Huy et al., 2019).

The dengue-specific IgM and IgG of this study were not assessed for their neutralization activity in DENV plaque reduction virus-cell culture assays, thus we cannot conclusively attribute the lower viremia in secondary infection than in primary infection to neutralization antibodies or removal of the virus by IgG in secondary infection. The viremia of DENV-1 higher than that of DENV-2 is consistent with previous reports of serotype-specific viremia (Soe et al., 2017; Vaughn et al., 2000). The detection of DENV-4 serotype in three mild cases of cohort 3 suggests that DENV-4 incidence is lower than DENV-1 and DENV-2 among symptomatic infections. The viral RNA detection in primary infection (82–97% NAT positive) and secondary infection (77–84% NAT positive) on day 0 hospitalization, within median days of first reported fever 2 or 3 days in primary infection and 4 or 5 days in secondary infection are consistent with the known dengue viremia lasting 4–5 days in the febrile phase (Nisalak, 2015). The differences in viral RNA detection between primary and secondary infection may in part be attributed to acute febrile cases in primary infection admitted to hospital earlier than acute febrile phase cases

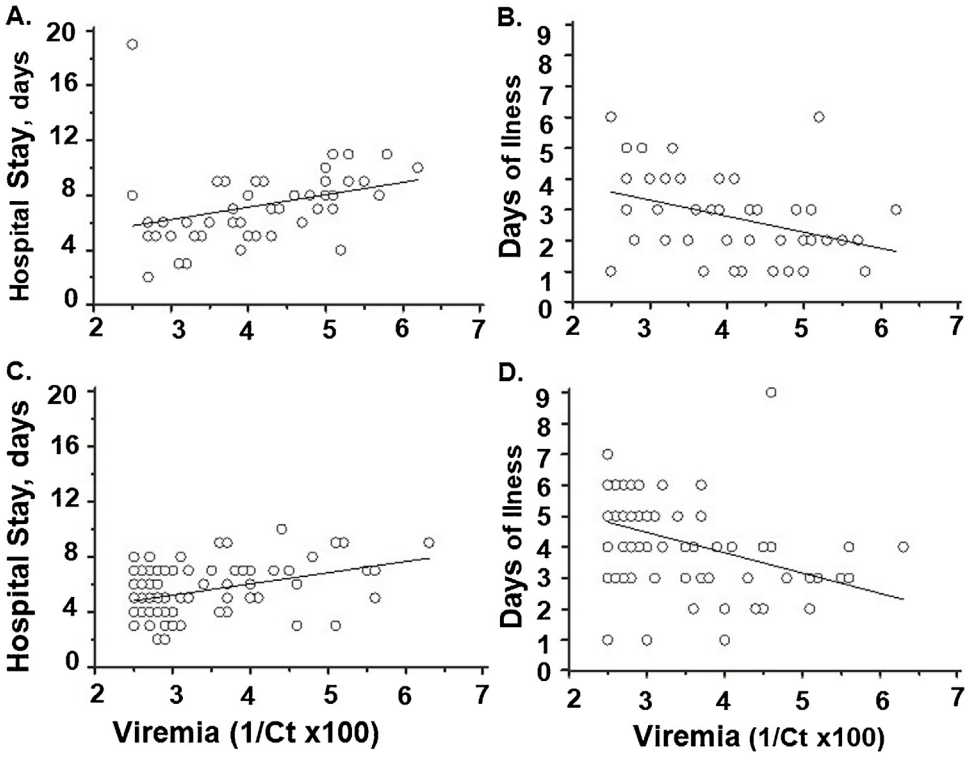

Figure 3. Associations of viremia with hospital stay and days of illness. The viremia versus hospital stay (A, C) and viremia versus days of illness (B, D) from combined data of cohorts 2 and 3. The coefficients of correlation in primary infection; r = 0.32 (95% CI 0.04–0.55) $p < 0.05$ (A) and r = −0.39 (95% CI −0.6 to −0.12), $p < 0.01$ (B) and the coefficients of correlation secondary infection; r = 0.4 (95% CI 0.22–0.56), $p < 0001$ (C) and r = −0.4 (95% CI 0.56–0.22), $p < 0.001$ (D).

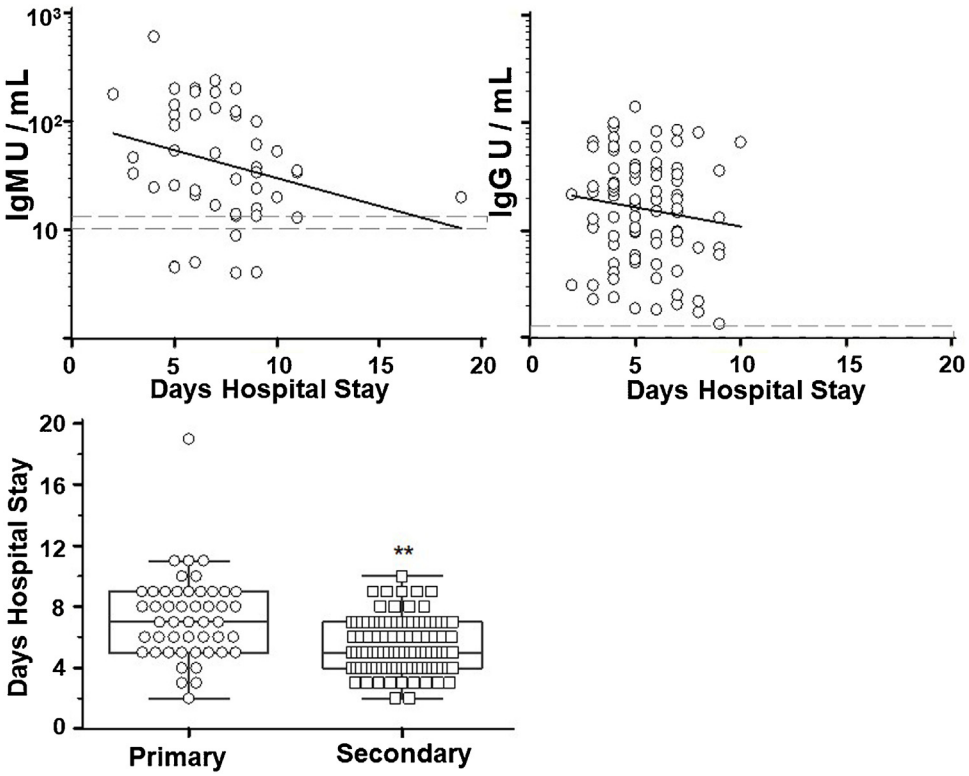

Figure 4. Anti-Dengue antibodies and hospital stay in primary versus secondary infection. IgM levels of primary infection versus hospital stay; r = −0.32 (95% CI −0,55 to −0,03), $p < 0.05$ (A), IgG levels of secondary infection versus hospital stay; r = −0.1(95% CI −0.3 to 0.1), p = 0.1 (B) and hospital stay in primary versus secondary dengue infection (C) from combined data of cohorts 2 and 3. ELISA cut off range of 10–15 U/mL within dotted rectangle. The difference between the median hospital stay in primary infection, 7 days (range 2–19 days) versus secondary infection; median 5 days (range 2–10 days) were of significance in the Mann–Whitney rank test **$p < 0.001$.

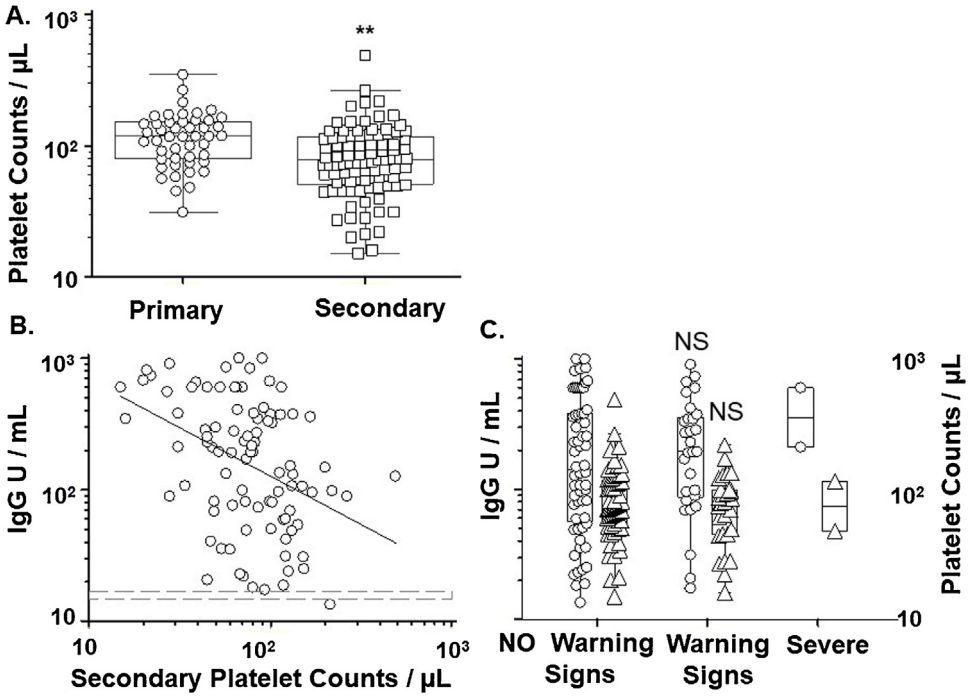

Figure 5. Platelet counts in primary versus secondary infection and association of platelet counts with IgG levels and dengue classification. Platelet counts from the combined data sets of cohort 2 and cohort 3; primary infection (open circles, N = 48); and secondary infection (open squares, N = 97) infection (A). IgG secondary infection versus platelet counts; r = −0.3 (95% CI −0.5 to −0.13), p < 0.01 (B). The IgG (open circles) and platelet counts (open triangles) in secondary infections are compared in dengue without warning signs; N = 60, dengue with warning signs; N = 29 and severe dengue N = 2 (C). **p < 0.01; the differences between the median platelet counts of primary versus secondary infection were of significance, NS; the differences between the median platelet counts or between the median IgG levels with or without warning signs were of no significance in Mann–Whitney rank test.

in secondary infection. To reduce false-positive results due to cross-reactivity with other Flaviviruses (Lee et al., 2019), the SERION IgG ELISA method minimizes yellow fever or Zika cross-reactivity by utilizing dengue serotype-specific epitopes of chimeric envelope protein III domain in the immunoglobulin capture (Batra et al., 2011). SERION ELISA *classic* dengue Virus IgM with inactivated viral lysate ensures the sensitive detection of specific IgM antibodies.

Since the reports of Zika in Vietnam are limited to sporadic cases among travellers in Ho Chi Minh city and Cambodian border with no reports in Hanoi (Dinh et al., 2019), the semi-quantitative IgM and IgG levels represent dengue-specific antibody responses of the primary and secondary infection which are likely not confounded by ZIka interference.

The mean IgM:IgG ratios in secondary infections of our study determined by ELISA, 1–1.8, are in the same range as the mean IgM:IgG ratios, 1.2–1.4 reported in plaque neutralization test (Raafat et al., 2019, Nguyen THT et al.; 2018). The IgM:IgG ratios at day 0, day 2 or 3 and day 7–10 hospitalization, reliably differentiate primary and secondary infection with IgM:IgG ratio cut-off 0.7 and are in line with the study in North India which reported IgM:IgG cut-off 1.1 to differentiate between primary and secondary infection (Changal et al., 2016).

Previous studies attribute the loss of platelet numbers and function in dengue to the depletion of tryptophan and serotonin by IFN-gamma IDO activity (Cui et al., 2016) or to autoantibodies generated from molecular mimicry between NS1 and platelet autoantigens (Jayathilaka et al., 2018). The link between the expression of anti-platelet autoantibodies and haemorrhagic inflammation and plasma leakage of mouse dengue strongly suggest a role for antiplatelet autoantibodies in the reduction of platelet numbers and function (Lien et al., 2015).

The study of 2017 dengue outbreak in Vietnam reported sustained platelet loss in adults within 7 days of their illness but did not distinguish between primary versus secondary infection (Huy et al., 2019). In this study, we confirm the platelet loss in adults and found significantly lower platelet counts in secondary infection than platelet counts in primary infection. Although anti-DENV IgG is implicated in the platelet loss of secondary infection (platelet counts negatively correlated with IgG levels), the IgG-associated platelet loss did not necessarily lead to a longer hospital stay as evident by the significantly lower median hospital stay in secondary infection than median hospital stay in primary infection. Additionally, the distribution of platelet counts in adult secondary dengue infection with warning signs were similar to the distribution of platelet counts without warning signs. For the above reasons we are cautious to interpret the platelet loss of secondary infection as a sign of worsening clinical outcomes of acute dengue infection but rather suggest as do previous studies that it may be an indicator for autoimmunity to manifest in adult post-dengue period (Chuang et al., 2014; Li et al., 2018; Rathnasiri-Bandara et al., 2019).

In conclusion, a high rate of secondary infection in Northern Vietnamese adults occurs with few severe complications. The overall good clinical outcomes of adult dengue are likely attributed to the young cases (median age under 40) without co-morbidities and the quick response of informed health care workers early in the outbreaks which are coordinated with correct and timely triage and management decision at the Hanoi hospitals. Future investigation of dengue infection in Vietnamese adults is warranted to identify autoantibodies and immunological markers after dengue recovery which can be linked to post-dengue autoimmune symptoms and complications.

Conflict of interests

The authors do not have a commercial or other association that might pose a conflict of interest. The Elisa Kits were provided by Serion GmbH.

Financial support

The study is funded by SeraDiaLogisticsand an internal grant from Vietnamese-German Center for Medical research.

Credit author statement

LH Song and NL Toan were principle investigators responsible for the EC-IRB applications and triage care of dengue cases at Hanoi hospitals. H Tong, CX Anh, H Hung, NX Hoan organized the blood specimens and the onsite collation of the clinical data. G Nematollahi, G Diez and T Schumacher developed and validated the ELISA tests and gave data analysis advice. D Fuchs gave laboratory support and O Landt performed the DENV nucleic acid testing. W Melchior helped conceptualize the study. TP. Velavan was instrumental in the VG CARE team management and review of the manuscript. SD Lytton conceptualized and initiated the study design, setup the on-site testing, analysed the data and wrote the draft manuscript.

Ethics approval

The study was approved by the Institutional Review Board of Vietnam Military Medical University (VMMU), Hanoi, Vietnam (Nr. 103MCH/RES/DENV-GER_V-D1-2016) and the 108 Hospital (Nr. Nr.108MCH/RES/DENV_D1-08-05-2018-SDL).

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