CLINICAL AND VACCINE IMMUNOLOGY, Dec. 2011, p. 2183–2185 Vol. 18, No. 12
1556-6811/11/$12.00 doi:10.1128/CVI.05425-11

Estimation of Dengue Virus IgM Persistence Using Regression Analysis▽

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Received 1 September 2011/Returned for modification 26 September 2011/Accepted 17 October 2011

Dengue virus IgM persistence was estimated using follow-up sera from 98 patients (60 with primary infections and 38 with secondary infections) whose first-specimen IgM index was strongly positive, suggesting recent disease onset. Regression analysis of the follow-up IgM index versus days between samples yielded a trend line that reached the cut-point index (1.10) at 179 days for the primary infection group and 139 days for the secondary infection group. This difference reflected significantly higher first-sample IgM indices in primary infections than in secondary infections rather than differences in IgM decay rates.

Dengue virus (DV) infections are transmitted to humans by *Aedes* sp. mosquitoes and are the leading cause of arbovirus-associated disease and death in tropical and subtropical locales throughout the world (2, 6, 15). Symptoms associated with primary infection by any of the 4 DV serotypes include fever, headache, and muscle and joint pains (6, 15). Subsequent infection with a different DV serotype (referred to as secondary infection) may result in the more serious forms of disease, dengue hemorrhagic fever and dengue shock syndrome (7, 12, 17).

Detection of DV IgM is an important laboratory tool for identifying patients with acute DV infection because of the relatively short time window wherein DV IgM is measurable (1, 3, 12). DV IgM reaches detectable levels in nearly all DV-infected patients within 5 days of symptom onset and reaches peak levels approximately 2 weeks later (1, 3, 5, 13, 14, 18). Peak IgM levels are usually lower in secondary infections than in primary infections (2–4, 16, 18). Although it is generally agreed that DV IgM wanes to undetectable levels within months of disease onset, published estimates of DV IgM persistence range from 2 months to 6 months (2, 4, 8, 16). We sought to estimate the time frame of DV IgM persistence by performing regression analysis of follow-up DV IgM results for patients whose initial specimen was strongly positive for DV IgM.

Procedures and materials. Serum levels of DV IgM were measured using a validated laboratory-developed IgM-capture enzyme-linked immunosorbent assay (ELISA) and DV IgG levels were measured using a validated laboratory-developed indirect ELISA as previously described (10). For each assay, index values of ≤1.10 were considered negative and values of >1.10 were considered positive. Sera received for DV IgM and IgG testing were not accompanied by data on symptoms, date of disease onset, or travel history. Patients whose first sample was DV IgM positive and who contributed another serum sample for DV IgM and IgG testing within a year were segregated into primary and secondary infection groups based on the IgM/IgG ratio of the first serum sample; as previously described (10), ratios of >1.32 were considered evidence of primary infection, whereas ratios of ≤1.32 were considered evidence of secondary infection.

The R package (11) was used to fit regression models to the logarithm of the second-serum IgM index as a function of the number of days between first and second samples for each patient. Additional models included an indicator variable that discriminated each patient's infection group (primary versus secondary). Statistical significance was defined as a P value of <0.05.

Results and conclusions. Because we did not have access to information regarding disease onset relative to specimen collection, we assumed that sera with high DV IgM index values came from DV-infected patients with a recent disease onset. A high DV IgM index was defined as >5.32, which was the median IgM index vale of 3,526 consecutive DV IgM-positive sera. We identified 98 patients who had a DV IgM index of >5.32 for their initial specimen and who also had a follow-up specimen collected within a year of the first specimen. For each patient, the IgM index of the second specimen was plotted as a function of the number of days between the first and second specimens (Fig. 1). The resulting regression trend line crossed the cut-point index discriminating positive results from negative results (1.10) at 160 days (95% conference interval, 139 to 193 days). These findings indicate that DV IgM persists for approximately 160 days (5.3 months) after first detection at a high index. Assuming that 1 to 2 weeks is required for a high index to be reached following disease onset (1, 3, 5, 13, 14, 18), we thus estimate that DV IgM persists for approximately 6 months after the onset of symptoms.

We next sought to determine if there was a difference in IgM persistence between patients with primary and secondary DV infections. Because DV IgM levels are typically higher following primary infection than following secondary infection (2–4, 16, 18), we hypothesized that it may take longer for IgM antibodies induced by primary infection to decay to negative levels. Patients were thus segregated into primary infection ($n = 60$) and secondary infection ($n = 38$) groups based on the first-sample IgM/IgG ratio; we recently demonstrated that, in our laboratory, a ratio of >1.32 was a reliable indicator of primary infection and a ratio of ≤1.32 was a reliable indicator

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▽ Published ahead of print on 26 October 2011.

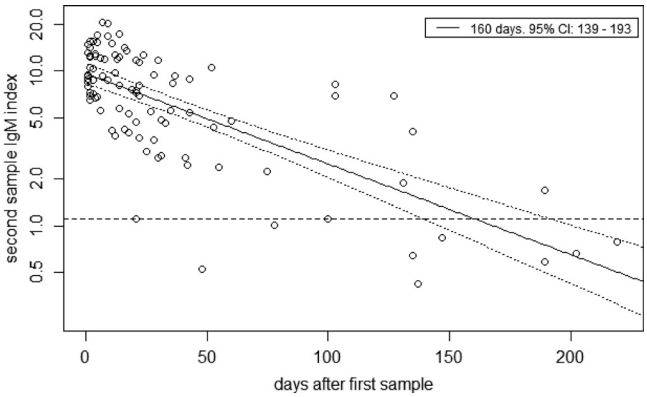

FIG. 1. Second-sample IgM index values plotted against the number of days between the first and second samples (range, 1 to 219 days) for 98 patients whose first sample was DV IgM positive with an index of >5.32. The solid line represents the trend line, and the dashed lines parallel to the trend line represent the 95% confidence interval. The horizontal dashed line indicates the IgM index value that discriminates positive from negative (1.10). The legend indicates the number of days until the trend line reached the discriminatory index and also the 95% confidence interval (CI).

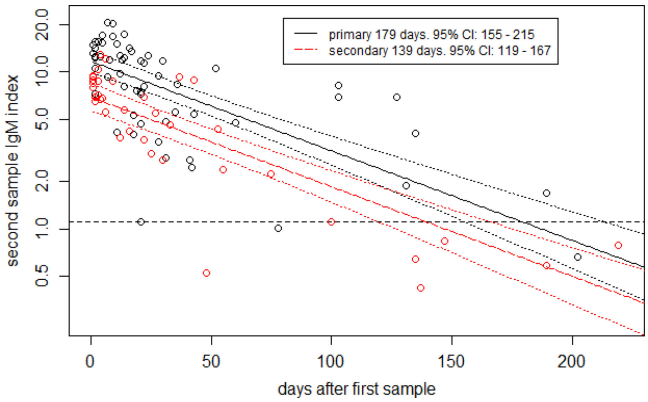

FIG. 2. Data from Fig. 1 segregated into primary and secondary infection groups based on IgM/IgG ratio. The line designations are the same as used in Fig. 1.

of secondary infection (10). Regression analysis showed that the trend line for the primary infection group reached the cut point at 179 days (95% confidence interval, 155 to 215 days), whereas the trend line for the secondary infection group reached the cut point at 139 days (95% confidence interval, 119 to 167 days) (Fig. 2).

Initial regression modeling indicated that the interaction between infection group and days after the initial visit was not statistically significant, based on the regression parameter's *t* test ($P = 0.13$); the logarithm of the IgM index decayed at a rate of 0.013 per day for both the primary infection and secondary infection groups. The difference in *y* intercept between the primary and secondary infection groups (IgM index values of 11.7 and 6.9, respectively) was statistically significant, consequently yielding different estimates for days to the cut-point index for each group. Based on this significant difference in *y* intercept for the primary and secondary groups as determined using follow-up specimens, we predicted that first-sample IgM indices would also significantly differ between the two groups; indeed, the mean first-sample IgM index (± standard deviation) for the primary infection group was 11.63 ± 3.98, compared to 7.77 ± 2.04 for the secondary infection group ($P < 0.001$). Taken together, these findings indicate that IgM persists longer following primary infection than following secondary infection, but the difference reflects higher starting levels of IgM in primary infection rather than a difference in the rate of IgM decay.

Our findings confirm and extend published information on DV IgM persistence. Nogueira et al. (8) found that IgM persisted in 49 of 50 patients tested within 90 days of disease onset. Similarly, our results support those of Vaughn et al. (16), who reported that the DV IgM levels for 45 patients had fallen to undetectable levels by 180 days after onset of fever. In contrast, our results differ from those of Gubler (2), who indicated that IgM waned to undetectable levels by 60 days in most patients.

We identified one published report of differences in DV IgM persistence between primary and secondary infections; Innis et al. (4) found that DV IgM was detectable in approximately 40% of primary infections but <10% of secondary infections at 60 days after disease onset. Thus, our findings concur with those of Innis et al. (4) from the standpoint of IgM persisting longer in primary infection than in secondary infection but differ from the standpoint of IgM duration. Our findings indicate that in both primary and secondary DV infections, IgM remains detectable for more than 60 days after disease onset.

Our study has some limitations. First, because clinical data were not available, we have assumed that the first sample for a given patient was collected within 1 to 2 weeks of disease onset. Thus, the strength of our estimate for DV IgM persistence, as well as the accuracy of primary and secondary infection assignments, may be compromised if we were incorrect in our assumption that high IgM levels in the first sample reflected more recent disease onset. Second, we cannot rule out the possibility that the DV antibodies detected in some patients were cross-reactive antibodies induced by infection or vaccination with another flavivirus, such as West Nile virus (WNV), Japanese encephalitis virus, or yellow fever virus (2, 6). Our previous studies (9) showed that WNV IgM persists for approximately 218 days, and thus, WNV-specific IgM may skew our estimation of DV IgM persistence. However, it seems unlikely that IgM induced by exposure to other flaviviruses would produce DV IgM index values >5.32 and thus qualify for inclusion in our analysis. Third, our estimates of DV IgM persistence are based on cross-sectional data from many patients; individual patient results will undoubtedly vary. A systematic assessment of IgM levels in serial specimens from patients with known disease onset dates, similar to that published for WNV-infected individuals (9), is needed to more accurately assess DV IgM persistence following acute infection.

The results presented here should prove useful in clinical settings where exposure to another flavivirus is suspected for a patient with recent DV infection. For example, a Colorado resident infected with DV in May (following a trip to India) and exhibiting symptoms consistent with WNV encephalitis in September would be expected to still have detectable DV IgM

in September, which could produce a false-positive result in a serologic assay for WNV IgM. This information should thus guide the physician to select additional laboratory tools for diagnosing WNV infection in this patient. Information on DV IgM persistence should prove helpful in other clinical settings as well. For example, for a patient from Puerto Rico who had a febrile illness in July consistent with DV infection (no laboratory evaluation performed) and who presents in October with a febrile illness, DV IgM detection would not necessarily be diagnostic for acute DV infection.

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