ORIGINAL ARTICLE

Effect of Dengue Serostatus on Dengue Vaccine Safety and Efficacy

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ABSTRACT

BACKGROUND

In efficacy trials of a tetravalent dengue vaccine (CYD-TDV), excess hospitalizations for dengue were observed among vaccine recipients 2 to 5 years of age. Precise risk estimates according to observed dengue serostatus could not be ascertained because of the limited numbers of samples collected at baseline. We developed a dengue anti–nonstructural protein 1 (NS1) IgG enzyme-linked immunosorbent assay and used samples from month 13 to infer serostatus for a post hoc analysis of safety and efficacy.

METHODS

In a case–cohort study, we reanalyzed data from three efficacy trials. For the principal analyses, we used baseline serostatus determined on the basis of measured (when baseline values were available) or imputed (when baseline values were missing) titers from a 50% plaque-reduction neutralization test ($PRNT_{50}$), with imputation conducted with the use of covariates that included the month 13 anti-NS1 assay results. The risk of hospitalization for virologically confirmed dengue (VCD), of severe VCD, and of symptomatic VCD according to dengue serostatus was estimated by weighted Cox regression and targeted minimum loss–based estimation.

RESULTS

Among dengue-seronegative participants 2 to 16 years of age, the cumulative 5-year incidence of hospitalization for VCD was 3.06% among vaccine recipients and 1.87% among controls, with a hazard ratio (vaccine vs. control) through data cutoff of 1.75 (95% confidence interval [CI], 1.14 to 2.70). Among dengue-seronegative participants 9 to 16 years of age, the cumulative incidence of hospitalization for VCD was 1.57% among vaccine recipients and 1.09% among controls, with a hazard ratio of 1.41 (95% CI, 0.74 to 2.68). Similar trends toward a higher risk among seronegative vaccine recipients than among seronegative controls were also found for severe VCD. Among dengue-seropositive participants 2 to 16 years of age and those 9 to 16 years of age, the cumulative incidence of hospitalization for VCD was 0.75% and 0.38%, respectively, among vaccine recipients and 2.47% and 1.88% among controls, with hazard ratios of 0.32 (95% CI, 0.23 to 0.45) and 0.21 (95% CI, 0.14 to 0.31). The risk of severe VCD was also lower among seropositive vaccine recipients than among seropositive controls.

CONCLUSIONS

CYD-TDV protected against severe VCD and hospitalization for VCD for 5 years in persons who had exposure to dengue before vaccination, and there was evidence of a higher risk of these outcomes in vaccinated persons who had not been exposed to dengue. (Funded by Sanofi Pasteur; ClinicalTrials.gov numbers, NCT00842530, NCT01983553, NCT01373281, and NCT01374516.)

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The first dengue vaccine — the recombinant, live, attenuated, tetravalent dengue vaccine (CYD-TDV) — was licensed on the basis of three efficacy trials in the Asia-Pacific region and Latin America.[1-3] After an excess of hospitalizations for dengue among children who had been vaccinated at 2 to 5 years of age was observed in the third year of the phase 3 trial in Asia (the CYD14 trial), the potential effects of baseline dengue serostatus and age on vaccine safety and efficacy required reconsideration.[4-6] One hypothesis for these excess cases was that CYD-TDV in recipients without previous dengue infection (i.e., dengue-unexposed vaccine recipients) mimics primary infection and, similar to natural secondary infection, places these people at an increased risk for severe disease on subsequent infection.[5,7]

The CYD-TDV efficacy trials assessed baseline dengue serostatus in a subset of participants (7.5% to 20%, depending on the trial) by measuring antibodies to each serotype with a 50% plaque-reduction neutralization test ($PRNT_{50}$). This limited subset, referred to as the immunogenicity subset, did not allow for precise estimates of the risk of hospitalization for dengue or the risk of severe dengue in seronegative vaccine recipients.[8] In an effort to overcome this limitation, blood samples that had been collected after the third vaccination were used to retrospectively determine baseline serostatus in a post hoc study. In the case–cohort study reported here, we used a newly developed dengue anti–nonstructural protein 1 (NS1) IgG enzyme-linked immunosorbent assay (ELISA)[9] to differentiate between anti-NS1 antibodies induced by wild-type dengue infection and those induced by vaccination (since CYD-TDV contains genes encoding NS1 from the yellow fever 17D vaccine virus rather than from dengue virus) to infer baseline dengue serostatus and reanalyze vaccine safety and efficacy according to serostatus.

METHODS

STUDY DESIGN

The findings of the CYD-TDV efficacy trials included in this analysis (CYD14 in the Asia-Pacific region, CYD15 in Latin America, and CYD23 [and its long-term follow-up extension study, CYD57] in Thailand) have been reported elsewhere.[1-3] The trials were similar to one another in design, with participants randomly assigned in a 2:1 ratio to the vaccine group or the control group at months 0, 6, and 12 and actively followed for disease to month 25. All participants who were randomly assigned to the control group in CYD14 and CYD15 received 0.9% saline placebo. In CYD23, all participants who were randomly assigned to the control group received 0.9% saline placebo, with the exception of the first 50 participants, who received inactivated rabies vaccine (Verorab, Sanofi Pasteur) for the first injection and 0.9% saline placebo for all other injections. Follow-up continued in order to monitor for disease leading to hospitalization (hospital phase), and active surveillance has subsequently been reinstated from approximately month 50 onward. Blood samples were collected during the acute phase of illness to virologically confirm dengue infection.

In this case–cohort study, we reassessed all cases of symptomatic virologically confirmed dengue (VCD), hospitalization for VCD, and severe VCD according to serostatus; this assessment included all cases occurring in participants in the immunogenicity subsets. From each trial, a subcohort of 10% of the participants was randomly selected after stratification according to age group and trial site. Details of the sampling strategy for the subcohort are provided in the analysis plan (Fig. S3 in the Supplementary Appendix, available with the full text of this article at NEJM.org). The case–cohort design provides power similar to that obtained with retesting of the entire cohort and enables efficient evaluation of multiple outcomes (Table SI in the Sample Size and Power Considerations section in the Supplementary Appendix).[10]

Sanofi Pasteur was the sponsor of the clinical trials and funded the work reported here and the development of the manuscript that was submitted. The sponsor was involved in all aspects of the trials and related analyses. The authors vouch for the accuracy and completeness of the data reported in this study.

ASSESSMENT METHODS

Month 13 anti-NS1 titers were measured in all participants in this case–cohort study for whom samples were available (Table S1 in the Supplementary Appendix). For the principal analyses,

dengue serostatus at the time of vaccination was defined on the basis of the baseline $PRNT_{50}$ serostatus as measured in the original trials (for participants with baseline values) or was imputed in analyses in which variables, including month 13 anti-NS1 titers as a continuous variable, were used as predictors (for participants in the case cohort with missing baseline values). Two imputation methods were used: logistic regression for multiple imputation and super learner[11] for targeted minimum loss–based estimation (see the Supplementary Appendix). For complementary analyses, cohorts were defined according to the month 13 anti-NS1 titer (month 13 NS1 method) with cutoff thresholds for positivity of 9 ELISA units (EUs) per milliliter or 20 EUs per milliliter. The threshold of 9 EUs per milliliter (the lower limit of quantitation) was chosen to minimize rates of false seronegative results.

END POINTS

The primary objective of the study was to assess the risk of hospitalization for VCD in seronegative vaccine recipients who were 9 years of age or older at enrollment (the primary end point). Prespecified secondary objectives included an assessment of this risk in seronegative participants in prespecified age groups (2 to 8 years and 2 to 16 years). The primary safety end point was hospitalization for VCD, and the other safety end point was severe VCD (as defined by an independent data and safety monitoring committee). Assessment of the efficacy of the vaccine against symptomatic VCD up to month 25 in prespecified age groups (2 to 8 years, 9 to 16 years, and 2 to 16 years) in seronegative participants was a secondary objective. Exploratory objectives included assessment of these end points among dengue-seropositive participants and a serotype-specific analyses. The definitions and methods of assessment of the safety and efficacy end points were the same as previously reported for the individual trials (see pages 9 through 12 in the Supplementary Appendix).[1-3]

STATISTICAL ANALYSIS

Cumulative incidence, hazard ratios, relative risks of severe VCD or hospitalization for VCD, and vaccine efficacy against symptomatic VCD were estimated on the basis of measured or imputed month 0 $PRNT_{50}$ serostatus (for the multiple imputation and targeted minimum loss–based estimation methods) and month 13 NS1 serostatus. In accordance with the original protocols (available at NEJM.org), efficacy was evaluated to month 25, and safety was evaluated over a long-term follow-up period (up to 6 years).

The multiple-imputation and NS1 methods involved a weighted Cox regression model with study group (vaccine or control) as a covariate. The regression models were not stratified according to serostatus or age group. Instead, separate subgroup analyses were performed with the use of the regression models for each category of serostatus and age group. Wald 95% confidence intervals of hazard ratios and P values were calculated.[12] With the multiple-imputation approach, estimates from 10 iterations were combined with the use of Rubin's variance rule.[13] Attributable risks were calculated as between-group differences in estimated cumulative incidence over 5 years. A parametric bootstrap approach (with 1000 samples) in the subcohort under the assumption of a Poisson distribution was used to estimate 95% confidence intervals for attributable risk. In analyses based on super learner to predict baseline serostatus, targeted minimum loss–based estimation was used to estimate cumulative incidences of dengue, from which vaccine efficacy, relative risk, and attributable risk were calculated.

Analyses of data from the individual trials and pooled data were conducted (phase 3 trials for efficacy and all trials for safety). Reported P values are two-sided. Although the prespecified analysis plan stated that P values were not going to be adjusted for multiplicity, we are presenting Holm–Bonferroni adjustment of P values for multiple safety end points, as well.[14]

We anticipated that imputation of baseline serostatus from month 13 anti-NS1 titers might be affected by dengue infection occurring between month 0 and month 13. Therefore, prespecified principal analyses were performed: from month 0 onward (including participants who had VCD between month 0 and month 13), and from month 13 onward (excluding participants who had VCD between month 0 and month 13). Our reasoning was based on the fact that the analysis from month 0 onward accounts for potential vaccine protection against events between month 0 and month 13 in cumulative efficacy

and risk estimates and maximizes the benefit of randomization. Complementary analyses in which month 13 NS1 serostatus was used assessed outcomes only from month 13 onward.

RESULTS

STUDY POPULATION

The case cohort included the 3578 participants in the subcohort (2384 in the vaccine group and 1194 in control group), as well as all the participants from the trials who had symptomatic VCD (1258 cases), hospitalization for VCD (644 cases), or severe VCD (142 cases) (Table S2 in the Supplementary Appendix). The distribution of baseline demographic characteristics in the overall, seropositive, and seronegative populations in the subcohort was balanced between the vaccine group and the control group; 24.5% of the participants were classified as seronegative by logistic regression (multiple-imputation), 24.0% by super learner (targeted minimum loss–based estimation), and 23.4% by measurement of anti-NS1 titers (with the cutoff of 9 EUs per milliliter). In the subcohort, data on month 0 $PRNT_{50}$ and month 13 anti-NS1 titers were missing for 66.7% (1591 of 2384) and 3.9% (93 of 2384), respectively, of vaccine recipients and 68.5% (818 of 1194) and 4.8% (57 of 1194) of controls. If (as is plausible when this sampling design is used) the probabilities of missing data are random after measured variables used in the analysis have been accounted for, then the inferences are expected to be valid. Additional details on the baseline characteristics of the participants and on missing data are provided in Tables S1, S3 through S6, and S46 in the Supplementary Appendix.

VALIDATION OF IMPUTED BASELINE DENGUE SEROSTATUS

The accuracy of the logistic-regression and super learner models was cross-validated for the predictability of measured baseline $PRNT_{50}$ serostatus; the methods produced similar results, with 79% of the participants who had been predicted to be seronegative by each method confirmed to be seronegative on the basis of measured $PRNT_{50}$ titers (Table S7 in the Supplementary Appendix). The analysis of concordance between the anti-NS1 titers at month 13 and $PRNT_{50}$ titers at month 0 and the analysis of the effect of CYD-TDV on the anti-NS1 titers at month 13 are provided in Table S8 and pages 19 through 23, respectively, in the Supplementary Appendix.

SAFETY AND EFFICACY ESTIMATES

Because the estimates calculated by the different analytic approaches were generally consistent with one another, pooled estimates based on the multiple-imputation approach (which is more commonly used for handling issues with missing data) from month 0 onward are reported unless indicated otherwise; estimates from all the methods we used are provided in the tables, figures, and Supplementary Appendix. Exploratory analyses showed statistical evidence of interaction between serostatus and treatment effect for the safety and efficacy end points ($P<0.01$ for all comparisons) and support the separate presentation of estimates for seronegative and seropositive populations. No statistical evidence of interaction between age groups (2 to 8 years and 9 to 16 years of age) and treatment effect on the safety end points in seronegative participants was observed in exploratory analyses, and therefore P values for these end points are reported only for the analyses involving participants 2 to 16 years of age.

Risk Associated with Vaccination at 9 to 16 Years of Age

Among seronegative participants 9 to 16 years of age, the hazard ratio (vaccine vs. control) for hospitalization for VCD was 1.41 (95% confidence interval [CI], 0.74 to 2.68) and that for severe VCD was 2.44 (95% CI, 0.47 to 12.56); the point estimates of the hazard ratio and relative risk were greater than 1 for all methods in the pooled analyses (Fig. 1, and Table S9 in the Supplementary Appendix). Through month 60 among seronegative participants, the cumulative incidence of hospitalization for VCD was 1.57% (95% CI, 1.13 to 2.19) in the vaccine group and 1.09% (95% CI, 0.53 to 2.27) in the control group, and the incidence of severe VCD was 0.40% (95% CI, 0.22 to 0.75) in the vaccine group and 0.17% (95% CI, 0.04 to 0.83) in the control group. Additional details of the results for seronegative participants, as well as exploratory analyses for each trial, are provided in Tables S10 through S12 in the Supplementary Appendix.

Among seropositive participants, the hazard ratio (vaccine vs. control) for hospitalization for

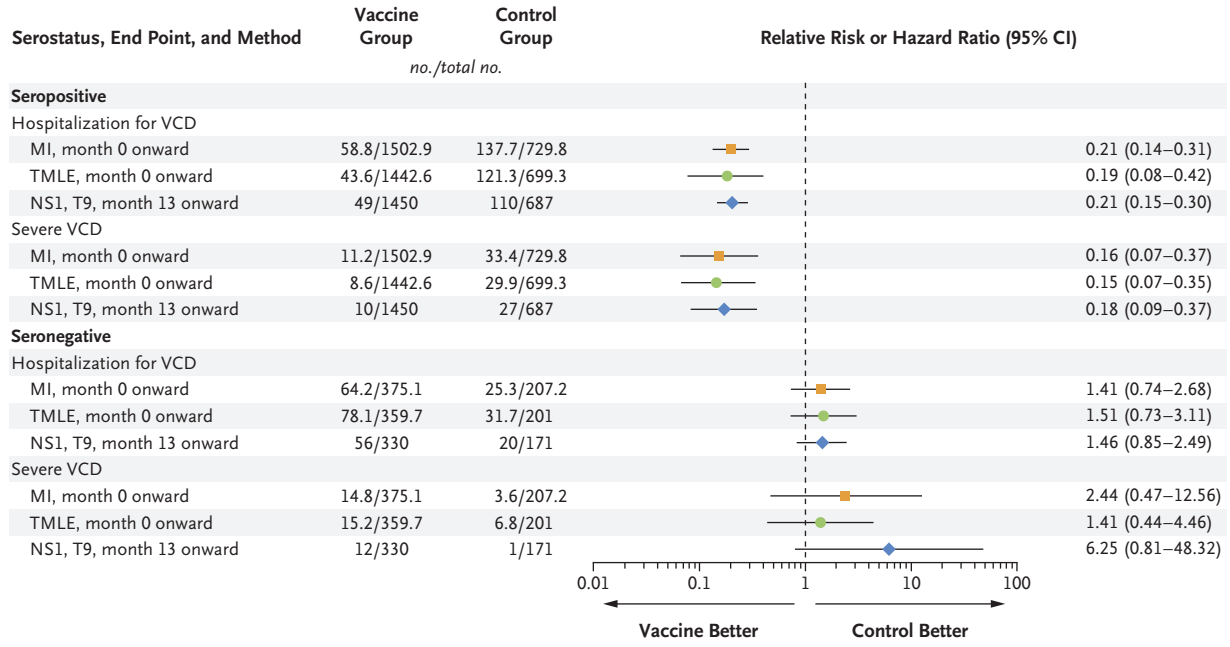


Figure 1. Risk of Hospitalization for Virologically Confirmed Dengue (VCD) and of Severe VCD in Participants 9 to 16 Years of Age, According to Baseline Serostatus.

Dengue serostatus was assigned on the basis of multiple imputation (MI, month 0 onward), targeted minimum loss–based estimation (TMLE, month 0 onward), and measured anti–nonstructural protein 1 (NS1) titer (threshold for positivity, 9 enzyme-linked immunosorbent assay [ELISA] units per milliliter [T9]; month 13 onward). Hazard ratios (MI and NS1) and relative risks (TMLE) are shown with corresponding 95% confidence intervals. For NS1, numerators represent the number of participants who were hospitalized for VCD or had severe VCD, and the denominators are the total numbers of participants selected in the subcohort. For MI, the numerators and denominators are the means of 10 iterations of MI, with the numerator representing the number of participants who were hospitalized for VCD or had severe VCD and the denominator representing the total number of participants selected in the subcohort. For TMLE, the numerators are the predicted numbers of study group–specific events among participants of the given serostatus within the subcohort, and the denominators are the predicted numbers of participants of a given serostatus within the subcohort. The analysis involved the as-treated population, in which participants were classified as being in the vaccine group if they had received at least one injection of CYD-TDV. Data were pooled from the CYD14, CYD15, and CYD23 (and CYD57) trials.

VCD was 0.21 (95% CI, 0.14 to 0.31) and that for severe VCD was 0.16 (95% CI, 0.07 to 0.37); the point estimates of the hazard ratios and relative risk were less than 1 with all methods in the pooled analyses (Fig. 1, and Table S9 in the Supplementary Appendix) and in the individual trials (Table S13 in the Supplementary Appendix). Table S14 in the Supplementary Appendix shows exploratory analyses involving seropositive participants who were 9 to 11 years and 12 to 16 years of age. Through month 60 among seropositive participants, the cumulative incidence of hospitalization for VCD was 0.38% (95% CI, 0.26 to 0.54) in the vaccine group and 1.88% (95% CI, 1.54 to 2.31) in the control group, and the cumulative incidence of severe VCD was 0.08% (95% CI, 0.03 to 0.17) in the vaccine group and 0.48% (95% CI, 0.34 to 0.69) in the control group (Table S10 in the Supplementary Appendix).

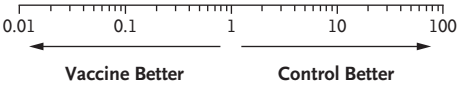
The attributable risk over a 60-month period per 1000 seronegative vaccine recipients was 4.78 (95% CI, –13.99 to 24.00) for hospitalization for VCD and 2.30 (95% CI, –7.00 to 10.67) for severe VCD. The corresponding attributable risk per 1000 seropositive vaccine recipients was –15.08 (95% CI, –25.44 to –4.97) and –4.05 (95% CI, –9.59 to 0.63), respectively.

Risk Associated with Vaccination at 2 to 8 Years of Age

Among seronegative participants 2 to 8 years of age, the hazard ratio (vaccine vs. control) for hospitalization for VCD was 1.95 (95% CI, 1.19

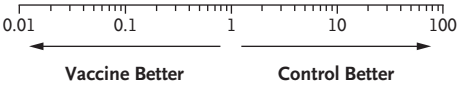
A 2–8 Yr of Age

Serostatus, End Point, and Method	Vaccine Group	Control Group	Relative Risk or Hazard Ratio (95% CI)
	no./total no.		
Seropositive			
Hospitalization for VCD			
MI, month 0 onward	93.6/313.2	89.8/156.4	0.50 (0.33–0.77)
TMLE, month 0 onward	55.6/299.7	78.2/147.4	0.35 (0.10–1.22)
NS1, T9, month 13 onward	75/300	65/135	0.53 (0.36–0.77)
Severe VCD			
MI, month 0 onward	23.9/313.2	20/156.4	0.58 (0.26–1.30)
TMLE, month 0 onward	19.6/299.7	20.0/147.4	0.45 (0.19–1.04)
NS1, T9, month 13 onward	21/300	17/135	0.56 (0.29–1.08)
Seronegative			
Hospitalization for VCD			
MI, month 0 onward	137.4/192.8	37.2/100.6	1.95 (1.19–3.19)
TMLE, month 0 onward	165.9/187.4	33.8/95.9	2.48 (0.64–9.54)
NS1, T9, month 13 onward	131/182	33/101	2.24 (1.43–3.50)
Severe VCD			
MI, month 0 onward	30.1/192.8	5/100.6	3.31 (0.87–12.54)
TMLE, month 0 onward	29.4/187.4	3.2/95.9	4.31 (0.80–23.15)
NS1, T9, month 13 onward	25/182	4/101	3.43 (1.17–10.09)



B 2–16 Yr of Age

Serostatus, End Point, and Method	Vaccine Group	Control Group	Relative Risk or Hazard Ratio (95% CI)
	no./total no.		
Seropositive			
Hospitalization for VCD			
MI, month 0 onward	152.4/1816.1	227.5/886.2	0.32 (0.23–0.45)
TMLE, month 0 onward	99.2/1742.3	199.5/846.7	0.25 (0.12–0.53)
NS1, T9, month 13 onward	124/1750	175/822	0.34 (0.26–0.43)
Severe VCD			
MI, month 0 onward	35.1/1816.1	53.4/886.2	0.31 (0.17–0.58)
TMLE, month 0 onward	28.2/1742.3	49.9/846.7	0.27 (0.15–0.48)
NS1, T9, month 13 onward	31/1750	44/822	0.33 (0.21–0.53)
Seronegative			
Hospitalization for VCD			
MI, month 0 onward	201.6/567.9	62.5/307.8	1.75 (1.14–2.70)
TMLE, month 0 onward	244.0/547.2	65.5/296.8	2.10 (0.94–4.70)
NS1, T9, month 13 onward	187/512	53/272	1.89 (1.35–2.65)
Severe VCD			
MI, month 0 onward	44.9/567.9	8.6/307.8	2.87 (1.09–7.61)
TMLE, month 0 onward	44.6/547.2	9.3/296.8	2.62 (1.03–6.70)
NS1, T9, month 13 onward	37/512	5/272	3.93 (1.53–10.10)



to 3.19) and that for severe VCD was 3.31 (95% CI, 0.87 to 12.54) (Fig. 2A); the point estimates were greater than 1 for all methods in pooled analyses and in individual trials. Among seropositive participants, the corresponding hazard ratios were 0.50 (95% CI, 0.33 to 0.77) and 0.58 (95% CI, 0.26 to 1.30) (Fig. 2A), and the point estimates were less than 1 in all analyses. Additional results, including the estimates of cumulative incidence and attributable risk and the results of exploratory analyses involving seronegative participants who were 2 to 5 years and

Figure 2 (facing page). Risk of Hospitalization for VCD and of Severe VCD in Participants 2 to 8 Years and 2 to 16 Years of Age, According to Baseline Serostatus.

Dengue serostatus was categorized on the basis of MI (month 0 onward), TMLE (month 0 onward), and NS1 (threshold for positivity, 9 ELISA units per milliliter [T9]; month 13 onward). Hazard ratios (MI and NS1) and relative risks (TMLE) are shown with corresponding 95% confidence intervals. For NS1, numerators represent the number of participants who were hospitalized for VCD or had severe VCD, and the denominators are the total numbers of participants selected in the subcohort. For MI, the numerators and denominators are means from 10 iterations of MI, with the numerator representing the number of participants who were hospitalized for VCD or had severe VCD and the denominator representing the total number of participants selected in the subcohort. For TMLE, the numerators are the predicted numbers of study group–specific events among participants of the given serostatus within the subcohort, and the denominators are the predicted numbers of participants of a given serostatus within the subcohort. The analysis involved the as-treated population, in which participants were classified as being in the vaccine group if they had received at least one injection of CYD-TDV. Data were pooled from the CYD14, CYD15, and CYD23 (and CYD57) trials. For the analysis involving participants who were 2 to 16 years of age, the unadjusted and adjusted P values for the comparisons between the vaccine group and the control group among seronegative participants were as follows: MI method for hospitalization for VCD, P=0.01 (unadjusted) and P=0.02 (Holm–Bonferroni adjusted); MI method for severe VCD, P=0.03 (unadjusted) and P=0.03 (Holm–Bonferroni adjusted); TMLE method for hospitalization for VCD, P=0.07 (unadjusted) and P=0.09 (Holm–Bonferroni adjusted); and TMLE method for severe VCD, P=0.04 (unadjusted) and P=0.09 (Holm–Bonferroni adjusted). Holm–Bonferroni adjustment was performed for the two safety end points (two tests) independently for each method.

6 to 8 years of age, are shown in Tables S11 and S14 through S18 in the Supplementary Appendix).

Risk Associated with Vaccination at 2 to 16 Years of Age

Among seronegative participants who were 2 to 16 years of age, the hazard ratio (vaccine vs. control) for hospitalization for VCD was 1.75 (95% CI, 1.14 to 2.70) and that for severe VCD was 2.87 (95% CI, 1.09 to 7.61) (Fig. 2B); all point estimates were greater than 1. Among seronegative participants, the cumulative incidence of hospitalization for VCD through month 60 was 3.06% (95% CI, 2.53 to 3.61) among vaccine recipients and 1.87% (95% CI, 1.23 to 2.86) among controls. Among seropositive participants, the corresponding hazard ratios were 0.32 (95% CI, 0.23 to 0.45) and 0.31 (95% CI, 0.17 to 0.58) (Fig. 2B), and point estimates were less than 1 in all analyses. Among seropositive participants, the cumulative incidence of hospitalization for VCD through month 60 was 0.75% (95% CI, 0.56 to 1.00) among vaccine recipients and 2.47% (95% CI, 2.09 to 2.92) among controls. Additional results are provided in Tables S19 through S22 in the Supplementary Appendix.

Risk over Time

Among seronegative participants, the hazard ratio (vaccine vs. control) for hospitalization for VCD was greater than 1 as estimated with most methods during the hospital phase (month 25 onward) in participants who were 9 to 16 years of age (Table S23 in the Supplementary Appendix). The risk estimates according to time period among participants who were 2 to 8 years or 2 to 16 years of age are shown in Tables S24 and S25 in the Supplementary Appendix. There was an excess risk of hospitalization for VCD in seronegative vaccine recipients as compared with seronegative controls from month 30 onward among those who were 9 to 16 years of age and from month 18 onward among those who were 2 to 8 years of age (Fig. 3). Among seropositive participants, the cumulative risk was lower in the vaccine group than in the control group throughout follow-up (Fig. 3, and Figs. S1 and S2 in the Supplementary Appendix).

Clinical Profile of Cases and Risk According to Dengue Virus Serotype

In all age groups, the median duration of fever, symptoms, and hospitalization did not differ between hospitalized patients with VCD in the vaccine group and those in the control group. A higher risk of plasma leakage and severe thrombocytopenia (platelet count, $<50\times10^9$ per liter) was found in the vaccine group (Table 1). The overall clinical picture among patients with severe VCD was similar in the two study groups, and most cases were dengue hemorrhagic fever (DHF) grade I or II, as defined in the World Health Organization (WHO) 1997 classification (Tables S26 and S27 in the Supplementary Appendix). All the affected participants recovered. There were no dengue-related deaths. The all-cause mortality rate in all the trials combined

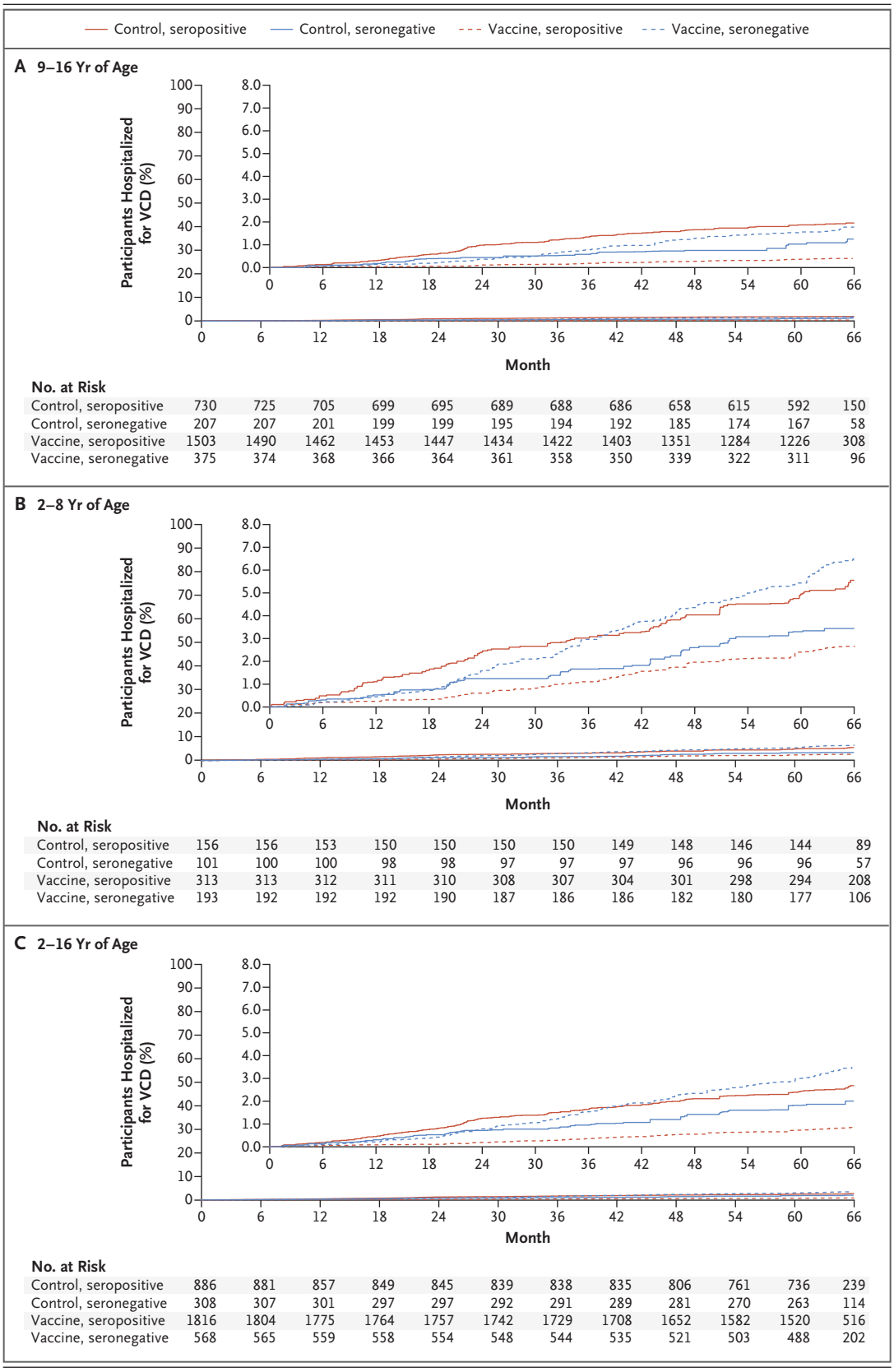


Figure 3 (facing page). Cumulative Incidence Curves of Hospitalization for VCD from Month 0 According to Baseline Serostatus as Classified by $PRNT_{50}$ at Baseline (Multiple-Imputation Approach) in Different Age Groups.

Data are from a pooled analysis of the CYD14, CYD15, and CYD23 (and CYD57) trials. The cumulative incidence curves are curtailed at month 66 to ensure that at least 20% of the participants remained at risk in each subcohort. Insets show the same data on an enlarged y axis.

was 0.24% (0.21% in the vaccine group and 0.30% in the control group), and no deaths from any cause were judged by the investigators and the sponsor to be related to the vaccine. No differences in the symptomatology between cases in seronegative vaccine recipients and those in seropositive controls were found (Tables S28 through S31 in the Supplementary Appendix). Among seronegative participants, the hazard ratio or relative risk (vaccine vs. control) of hospitalization for VCD caused by serotype 1 or 3 dengue virus was greater than 1 in some analyses, and these ratios were consistently greater than 1 for hospitalization for VCD caused by serotype 2 dengue virus. Among seropositive participants, the hazard ratios and relative risks of hospitalization for VCD were less than 1 for dengue due to each of the four serotypes (Table S32 and S33 in the Supplementary Appendix).

Vaccine Efficacy against Symptomatic VCD

Among seronegative participants, vaccine efficacy against symptomatic VCD (up to month 25) was 39% (95% CI, −1 to 63) among those who were 9 to 16 years of age, 19% (95% CI, −47 to 55) among those who were 2 to 8 years of age, and 32% (95% CI, −9 to 58) among those who were 2 to 16 years of age. Among seropositive participants, the corresponding values were 76% (95% CI, 64 to 84), 60% (95% CI, 31 to 76), and 73% (95% CI, 59 to 82) (Fig. 4). Vaccine efficacy as determined by all other methods and according to trial, serotype, and age stratum are shown in Tables S34 through S43 in the Supplementary Appendix.

DISCUSSION

Using a new assay and several analytic methods, we reanalyzed serum samples from three efficacy trials to further characterize the safety and efficacy of CYD-TDV according to dengue serostatus at the time of vaccination. We found that among dengue-seropositive participants, vaccination conferred protection against subsequent disease for at least 5 years; the rates of severe VCD and hospitalization for VCD over a 5-year period for all the ages considered (2 to 16 years of age) were approximately 70% lower in the vaccine group than in the control group, and among those 9 years of age or older at vaccination (the vaccine-indicated age group), the rates of severe VCD and hospitalization for VCD were approximately 80% lower in the vaccine group than in the control group.

Among dengue-seronegative participants, however, over the same period, the rates of hospitalization for VCD and of severe VCD were higher in the vaccine group than in the control group. A trend toward a higher risk of hospitalization for VCD in association with vaccination was found among seronegative participants who were 9 to 16 years of age, and a significantly higher risk was found among seronegative participants who were 2 to 8 years of age. Among seronegative vaccine recipients 9 to 16 years of age, the onset of a higher risk of hospitalization for VCD occurred mainly during the third year after the first vaccination, whereas among younger seronegative vaccine recipients, the higher risk seemed to start earlier. Our findings indicate a major role for previous dengue exposure in modifying vaccine performance and provide some evidence of a possible age effect. However, since age is associated with dengue exposure, it remains unclear whether these findings reflect undetected dengue exposure that was not captured by the assays (i.e., false seronegatives) or age-specific differences after vaccination or infection.[15]

Most participants did not have severe VCD during the trial; the rates of severe VCD were low among both seronegative vaccine recipients and seropositive controls, which indicated the rarity of severe dengue even in areas of high endemicity. On the basis of our data, the population-level effect of vaccination would be an excess of hospitalizations for dengue and of severe cases in seronegative persons, as well as a lower rate of these events among seropositive persons. Extrapolating the estimates of attributable risk to a cohort of 1 million people 9 to 16 years of age with an 80% rate of seropositivity suggests that, over a period of 5 years, vaccination would

Table 1. Clinical Signs and Symptoms in All Hospitalizations for Virologically Confirmed Dengue (VCD) Occurring from Month 13 to the End of the Follow-up Period (Month 60 to Month 72) among Seronegative Participants.*

Outcome	2–16 Yr			9–16 Yr			2–8 Yr		
	Vaccine Group (N=512)	Control Group (N=272)	Risk Ratio (95% CI)†	Vaccine Group (N=330)	Control Group (N=171)	Risk Ratio (95% CI)†	Vaccine Group (N=182)	Control Group (N=101)	Risk Ratio (95% CI)†
No. of hospitalizations for VCD	188	54	1.89 (1.35–2.65)‡	56	20	1.46 (0.85–2.49)‡	132	34	2.24 (1.43–3.50)‡
Median duration of clinical symptoms (range) — days	8 (3–29)	7.5 (3–18)		8 (3–29)	7.5 (4–14)		8 (4–18)	7.5 (3–18)	
Median duration of fever (range) — days	5 (1–10)	5 (2–11)		5 (1–10)	5 (2–8)		5 (2–10)	5 (2–11)	
No. of hospitalizations according to serotype									
Serotype 1	62	23		24	9		38	14	
Serotype 2	71	11		19	5		52	6	
Serotype 3	38	13		11	6		27	7	
Serotype 4	15	8		3	2		12	6	
Median duration of hospitalization (range) — days	4 (1–11)	4 (2–7)		4 (1–8)	4 (2–6)		5 (2–11)	4 (2–7)	
Any hemorrhage — no. of cases/total no. with data (%)	79/188 (42.0)	24/54 (44.4)	0.94 (0.59–1.56)	22/56 (39.3)	9/20 (45.0)	0.87 (0.39–2.15)	57/132 (43.2)	15/34 (44.1)	0.98 (0.55–1.86)
Any visceral manifestation — no. of cases/total no. with data (%)	4/188 (2.1)	1/54 (1.9)	1.15 (0.11–56.58)	0/56	1/20 (5.0)	0.00 (0.00–13.93)	4/132 (3.0)	0/34	
Plasma leakage — no. of cases/total no. with data (%)									
Any	67/188 (35.6)	7/54 (13.0)	2.75 (1.26–7.10)	20/56 (35.7)	2/20 (10.0)	3.57 (0.87–31.51)	47/132 (35.6)	5/34 (14.7)	2.42 (0.97–7.80)
With clinical signs	11/188 (5.9)	1/54 (1.9)	3.16 (0.46–136.00)	2/56 (3.6)	0/20		9/132 (6.8)	1/34 (2.9)	2.32 (0.32–101.61)
Hematocrit increase ≥20%	66/188 (35.1)	7/54 (13.0)	2.71 (1.24–7.00)	20/56 (35.7)	2/20 (10.0)	3.57 (0.87–31.51)	46/132 (34.8)	5/34 (14.7)	2.37 (0.95–7.64)
Thrombocytopenia — no. of cases/total no. with data (%)									
Platelet count $\leq 50\times10^9$/liter	71/188 (37.8)	7/54 (13.0)	2.91 (1.34–7.51)	23/56 (41.1)	3/20 (15.0)	2.74 (0.83–14.25)	48/132 (36.4)	4/34 (11.8)	3.09 (1.13–11.80)
Platelet count $\leq 100\times10^9$/liter	141/188 (75.0)	26/54 (48.1)	1.56 (1.02–2.47)	43/56 (76.8)	14/20 (70.0)	1.10 (0.59–2.17)	98/132 (74.2)	12/34 (35.3)	2.10 (1.15–4.21)
Shock — no. of cases/total no. with data (%)	3/187 (1.6)	0/52		0/56	0/20		3/131 (2.3)	0/32	

* Dengue serostatus was determined on the basis of the measured anti-NS1 titer (threshold for positivity, 9 enzyme-linked immunosorbent assay [ELISA] units per milliliter) at month 13. N denotes the total number of participants in the subcohort for each age group and study group. The data are from a pooled analysis of the CYD14, CYD15, and CYD23 (and CYD57) trials.

† The risk ratio is calculated as the ratio of the number of cases with the specified clinical sign or symptom in the vaccinated group as compared with the control group among the participants who were hospitalized for VCD.

‡ Values are hazard ratios for hospitalization for VCD in seronegative participants and are based on NS1 serostatus (month 13 onward; threshold for positivity, 9 ELISA units per milliliter).

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prevent approximately 11,000 hospitalizations (12,000 avoided among seropositive persons with 1000 excess among seronegative persons) and approximately 2500 severe cases (3000 avoided among seropositive persons and 500 excess among seronegative persons). However, these numbers should be interpreted cautiously, since they reflect the epidemiologic contexts of the clinical trials and are expected to differ in other contexts and over time (because factors affecting population-level vaccine performance, such as the baseline rate of dengue seropositivity, force of infection, and incidence of infection, may change). Dynamic transmission models can help provide an understanding of the interactions among incidence, seroprevalence, and time-dependent factors.[16,17]

Symptomatic, nonsevere dengue is an important condition with a substantial outpatient burden.[18] Among seropositive persons, we found high efficacy of the vaccine against symptomatic VCD (up to month 25), with low-to-modest efficacy suggested among seronegative vaccine recipients. These findings are consistent with previous observations based on measured $PRNT_{50}$ titers at baseline in the immunogenicity subset from the trials.[4]

Our findings support the hypothesis that, in the absence of previous dengue exposure, the CYD-TDV vaccine partially mimics primary infection and increases the risk of severe dengue during subsequent infection, similar to the risk that is observed epidemiologically in association with a natural second dengue infection. One notable difference is that the risk of natural secondary dengue infection is associated with naturally acquired monotypic antibodies, whereas the observed risk with CYD-TDV vaccination occurs after induction of multitypic antibody responses. Nevertheless, the pattern of risk we found is consistent with the previous hypothesis of a clustering of the risk related to vaccination time.[5] We found no meaningful clinical differences in the symptomatology of severe cases among seronegative vaccine recipients (37 cases among those who were 2 to 16 years of age), seronegative controls (5 cases), and seropositive controls (44 cases); the small numbers make these comparisons fragile at best.

The immunopathogenic mechanisms underlying these findings remain unknown. Although antibody-dependent enhancement has been proposed as a mechanistic basis of the increased risk of severe dengue associated with a subsequent second dengue infection,[19] and recent evidence from a longitudinal cohort study of natural dengue infection suggests an increased risk in the presence of low antibody titers,[20] our study did not specifically investigate whether antibody-dependent enhancement, other pathogens, or host or environmental factors played a role. However, these observations may be partly explained by differences in vaccine performance according to dengue serotype.

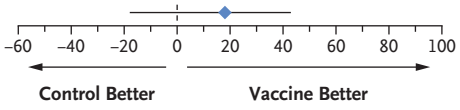
Overall, the general consistency of the results obtained with different analytic methods supports our findings. However, each approach relies on assumptions. Both multiple imputation and targeted minimum loss–based estimation rely on the "missing-at-random" assumption, which, although unverifiable, we think is likely to hold by design.[21,22] Multiple imputation has well established operating characteristics but relies on correctly specifying a model for the month 0 serostatus probabilities. Targeted minimum loss–based estimation is "doubly robust" in that it incorporates both the conditional month 0 serostatus probability and the probability of having missing data while relying on only one of these probabilities being correctly specified. However, in relying on weaker assumptions, it can produce larger standard errors than multiple imputation.

Unlike multiple imputation and targeted minimum loss–based estimation, the month 13 NS1 method uses a measured proxy for month 0 serostatus that does not rely on correct model specification for the missing data. However, the categorization of month 13 NS1 serostatus according to a defined threshold is subject to differential misclassification and underestimation of vaccine efficacy and cannot account for events that occurred before month 13 (see pages 23 through 26 in the Supplementary Appendix). Our cross-validation results indicate that both logistic regression and super learner were highly predictive of observed month 0 serostatus. Although serostatus predictions were most accurate for analyses that excluded events that occurred from month 0 to month 13, analyses from month 0 onward account for vaccine effects between month 0 and month 13 and preserve the benefits of randomization.

Beyond the methodologic considerations mentioned, there are some important caveats: although we used a new assay after characterization and

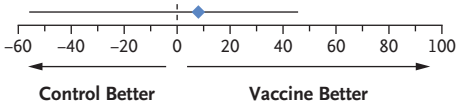
A 9–16 Yr of Age

Serostatus, End Point, and Method	Vaccine Group	Control Group	Vaccine Efficacy (95% CI)
	no./total no.		%
Seropositive			
Symptomatic VCD			
MI, month 0 onward	192.7/1441.4	372.1/697.3	76 (64 to 84)
TMLE, month 0 onward	175.1/1384.4	317.9/663.7	74 (66 to 80)
NS1, T9, month 13 onward	111/1389	222/656	77 (70 to 82)
Seronegative			
Symptomatic VCD			
MI, month 0 onward	174.3/353.6	148.9/193.7	39 (–1 to 63)
TMLE, month 0 onward	187.9/338.7	194.1/196	45 (26 to 58)
NS1, T9, month 13 onward	104/309	62/157	18 (–18 to 43)



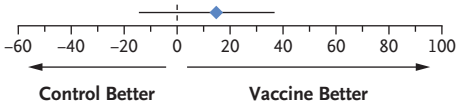
B 2–8 Yr of Age

Serostatus, End Point, and Method	Vaccine Group	Control Group	Vaccine Efficacy (95% CI)
	no./total no.		%
Seropositive			
Symptomatic VCD			
MI, month 0 onward	88.5/217.3	104.3/109.5	60 (31 to 76)
TMLE, month 0 onward	71.8/211.7	80.8/103.3	57 (38 to 70)
NS1, T9, month 13 onward	30/209	42/91	70 (49 to 82)
Seronegative			
Symptomatic VCD			
MI, month 0 onward	107.5/141.7	69.7/74.5	19 (–47 to 55)
TMLE, month 0 onward	103.2/141.6	76.2/73.4	28 (–2 to 48)
NS1, T9, month 13 onward	49/128	29/73	8 (–56 to 46)



C 2–16 Yr of Age

Serostatus, End Point, and Method	Vaccine Group	Control Group	Vaccine Efficacy (95% CI)
	no./total no.		%
Seropositive			
Symptomatic VCD			
MI, month 0 onward	281.2/1658.7	476.4/806.8	73 (59 to 82)
TMLE, month 0 onward	246.9/1596	398.6/767	71 (64 to 76)
NS1, T9, month 13 onward	141/1598	264/747	75 (69 to 80)
Seronegative			
Symptomatic VCD			
MI, month 0 onward	281.8/495.3	218.6/268.2	32 (–9 to 58)
TMLE, month 0 onward	291.1/480.3	270.4/269.3	40 (25 to 52)
NS1, T9, month 13 onward	153/437	91/230	15 (–15 to 37)



qualification, neither the anti-NS1 assay nor the PRNT is validated or registered for the purpose of determining baseline dengue serostatus. Serostatus classification relies heavily on the performance characteristics of the assays used, as well as on pretest probabilities of the condition of

Figure 4 (facing page). Vaccine Efficacy against Symptomatic VCD up to Month 25 According to Baseline Serostatus in Different Age Groups.

Dengue serostatus was categorized on the basis of MI (month 0 onward), TMLE (month 0 onward), and NS1 (threshold for positivity, 9 ELISA units per milliliter [T9]; month 13 onward). Vaccine efficacy estimates are shown with corresponding 95% confidence intervals. For NS1, the numerators represent the number of participants who had symptomatic VCD and the denominators represent the total participants selected in the subcohort; estimates are from month 13 to month 25. For MI, the numerators and denominators are means from 10 iterations of MI, with the numerator representing the number of participants with symptomatic VCD and the denominator representing the total number of participants selected in the subcohort; estimates are from month 0 to month 25. For TMLE, the numerators are the predicted numbers of study group–specific events among participants of the given serostatus within the subcohort and the denominators are the predicted numbers of participants of a given serostatus within the subcohort; estimates are from month 0 to month 25. The analysis involved the intention-to-treat population, with participants included in the group (vaccine or control) to which they had been randomly assigned. Data were pooled from the CYD14 and CYD15 trials.

interest. Therefore, predictive values are expected to vary in different epidemiologic contexts. In this post hoc study, power was predetermined by the number of cases that had been observed in the original trials, and small-to-moderate effects might have been missed because of the limited numbers of some safety end points.

Our findings could affect the implementation of dengue vaccination programs. In December 2017, the WHO published an interim position addressing this new information, and the WHO Strategic Advisory Group and Experts (SAGE) made recommendations available after their April 2018 meeting.[23,24] A reliable, rapid test to determine previous dengue exposure would be ideal; however, no such test has been widely registered for this indication, and prevaccination screening in large programs could be challenging to implement.

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APPENDIX

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