क्षेत्रीय आयुर्विज्ञान संस्थान, इंफाल: मणिपुर
REGIONAL INSTITUTE OF MEDICAL SCIENCES, IMPHAL, MANIPUR
(स्वास्थ्य और परिवार कल्याण मंत्रालय,भारत सरकार के अंतर्गत एक स्वायत्त संस्थान)
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CIRCULAR

Imphal, the 17th February, 2026

No. 35/RIMS-MRU/2026: It is to notify that, the **10th Research Masterclasses 2026**, of the Department of Health Research, Ministry of Health and Family Welfare, Government of India, will be conducted virtually, **on 6th March, 2026 (Friday)**.

2. All the faculties (RIMS, Dental College, and College of Nursing), members of EC, LRAC of MRU, Principal Investigators undertaking MRU funding projects (including under process projects) and residents are invited to attend the session at **Banting Hall, RIMS, Imphal**.

Date: 06.03.2026 (Friday)
Time: 3:00 PM onwards
Venue: Banting Hall, RIMS, Imphal (DESIGNATED SITE FOR PARTICIPATION FOR RIMS)
Event name: Research Masterclass under DHR-ICMR Research Grand Rounds
Speaker Name: Prof. Rakesh Lodha, Division of Pulmonology, Intensive Care, Tuberculosis & Infectious Diseases, Department of Paediatrics, All India Institute of Medical Sciences, New Delhi.

3. The **research paper** to be discussed during the Masterclass will be uploaded on the **RIMS website** and circulated to the concerned Departments/Colleges through official email.

4. As per the directives issued by the DHR, **maximum participation** from our institute is highly encouraged. MRU is submitting the attendance sheet to the DHR after the session concludes.

17-02-26

Prof. T. Jeetenkumar Singh,
Nodal Officer,
Multi-Disciplinary Research Unit,
RIMS, Imphal

Copy to:

1. The P.S. to Director, RIMS, for kind information of Director
2. The P.A. to Medical Superintendent, RIMSH, for kind information
3. The Dean (Academic), RIMS, for kind information & permission to utilize the facilities at Banting Hall, RIMS.
4. The Principal, Dental College, RIMS
5. The Principal, College of Nursing, RIMS
6. The Head of Department, RIMS, Imphal

   — with a request for ensuring maximum participation.
7. The Chairperson/Co-Chairperson/Member, LRAC, MRU, RIMS.....
8. The Member, EC, MRU, RIMS, Imphal.....
9. The Principal Investigator, RIMS .....
10. The IT Cell, RIMS – with a request for uploading the notice in the website & technical support on **06.03.26**
11. Asst. Engineer (Elect. /Civil), RIMS - with a request for ensuring uninterrupted power supply & optimum AC functioning.
12. The Care Taker, Banting Hall, RIMS, Imphal- for proper upkeep of the venue & the accompanying facilities.
13. Guard file.

No. R.11016/03/2025-HR
भारत सरकार/Government of India
स्वास्थ्य एवं परिवार कल्याण मंत्रालय/Ministry of Health & Family Welfare
स्वास्थ्य अनुसंधान विभाग/Department of Health Research

2nd Floor, IRCS Building
Sansad Marg, New Delhi – 110001
Dated 16.02.2026

To
The Dean/ Principal/ Director of Medical Colleges/ Institutes

Subject: Request to attend Research Masterclasses for MRU network– reg.

Sir/Madam,

DHR-ICMR has initiated a dedicated platform to conduct Research Grand Rounds to strengthen the National research ecosystem through sustained collaboration and knowledge exchange. The objectives of the Research Grand Rounds are as follows:

I. To deliberate on research methodologies, analytical tools, and emerging scientific approaches
II. To strengthen the methodological understanding amongst researchers needed to implement different kinds of research.
III. To foster collaboration and connectivity across research institutions

2. These Research Grand Rounds will be organized as monthly webinars entitled 'Research Masterclass' proposed around the last Friday of each month. The speakers for these Research Masterclasses will be eminent research scientists in the country who will be discussing their original research work in details from methodological point of view.

3. The next Research Masterclass is scheduled for **06.03.2026 (Friday)** at **3:00 PM**. The invited speaker is **Prof. (Dr.) Rakesh Lodha, Division of Pulmonology, Intensive Care, Tuberculosis & Infectious Diseases**, Department of Paediatrics, All India Institute of Medical Sciences, New Delhi. The research paper to be discussed during the research masterclass is enclosed. The link for the research masterclass will be shared shortly.

4. Accordingly, it is requested to kindly disseminate the information in your institution and ensure maximum participation in Research Masterclass. Your institute is requested to share at least two questions related to research paper attached on the following email: **dhr-mru@gov.in** latest by 28.02.2026. These questions will be discussed with the speaker during masterclass.

Yours faithfully,

(Dharkat R. Luikang)
Deputy Secretary to the Govt. of India

Copy to: The Nodal Officer of Multi-Disciplinary Research Units (MRUs)

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Brief Communication

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Severe disease during both primary and secondary dengue virus infections in pediatric populations

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A list of authors and their affiliations appears at the end of the paper

Dengue is a global epidemic causing over 100 million cases annually. The clinical symptoms range from mild fever to severe hemorrhage and shock, including some fatalities. The current paradigm is that these severe dengue cases occur mostly during secondary infections due to antibody-dependent enhancement after infection with a different dengue virus serotype. India has the highest dengue burden worldwide, but little is known about disease severity and its association with primary and secondary dengue infections. To address this issue, we examined 619 children with febrile dengue-confirmed infection from three hospitals in different regions of India. We classified primary and secondary infections based on IgM:IgG ratios using a dengue-specific enzyme-linked immunosorbent assay according to the World Health Organization guidelines. We found that primary dengue infections accounted for more than half of total clinical cases (344 of 619), severe dengue cases (112 of 202) and fatalities (5 of 7). Consistent with the classification based on binding antibody data, dengue neutralizing antibody titers were also significantly lower in primary infections compared to secondary infections ($P \leq 0.0001$). Our findings question the currently widely held belief that severe dengue is associated predominantly with secondary infections and emphasizes the importance of developing vaccines or treatments to protect dengue-naive populations.

Dengue infections have greatly increased in India during the past two decades and India now has the largest number of dengue cases globally[1]. However, not much is known about the proportion of primary versus secondary dengue infections and how this correlates with disease severity. In this study, we examined children with confirmed febrile dengue from three hospitals in different regions of India. Three tertiary care centers in India—St. John's Research Institute (SJRI), All India Institute of Medical Sciences (AIIMS) and Christian Medical College (CMC)—participated in this study, where 619 children with confirmed dengue were studied between 2012 and 2018. The characteristics of these patients are shown in Extended Data Table 1. Recruitment was done from 2014 to 2016 (SJRI), 2012 to 2018 (AIIMS) and 2015 to 2017 (CMC). The age of the children ranged from 2 months to 16 years; all the sites included both males and females. The infecting dengue virus serotype was identified in about 65% of patients and infections were seen with Dengue virus 1 (DENV-1) ($n = 188$), DENV-2 ($n = 143$), DENV-3 ($n = 55$) and DENV-4 ($n = 8$). Patients with confirmed dengue were classified as either primary or secondary dengue infection based on the ratio of the index value of dengue-specific plasma IgM and IgG using standard-capture enzyme-linked immunosorbent assays (ELISAs) (Panbio) as per the World Health Organization (WHO) guidelines[2]. Analysis of primary versus secondary infection status at all three clinical sites showed that patients consisted of a mix of primary (344 of 619) and secondary (275 of 619) infections (Extended Data Table 2). This mix of primary and secondary infections was seen at each of the individual sites.

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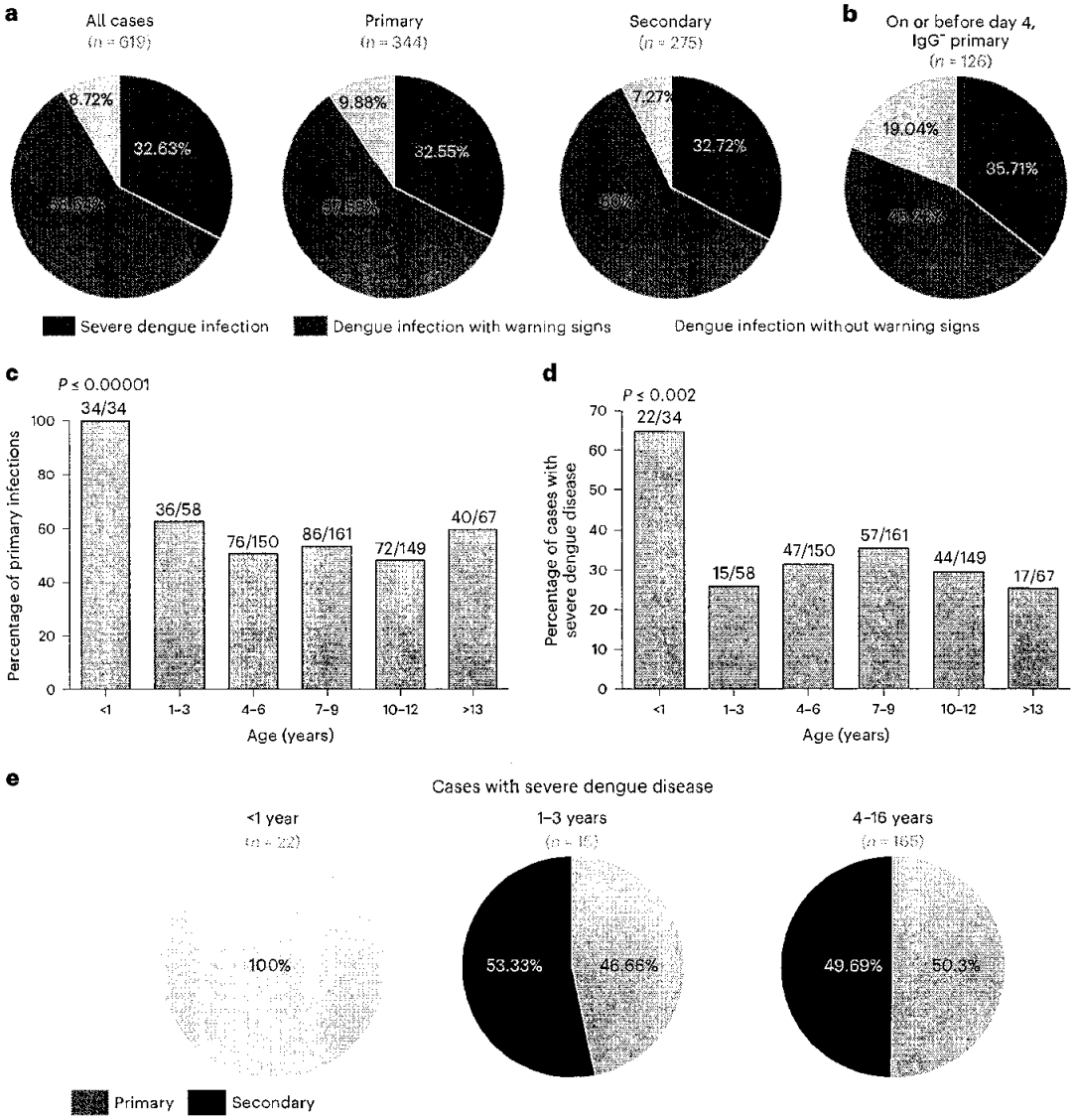

Fig. 1 | Similar frequency of severe disease in pediatric patients with primary versus secondary dengue infections. a, Frequency of severe dengue infection, dengue infection with warning signs or dengue infection without warning signs cases among children with confirmed dengue (all cases, $n = 619$) and among those classified as primary ($n = 344$) or secondary ($n = 275$) dengue infection as described in the Methods. Disease severity was not significantly different between primary and secondary cases ($P = 0.53$, Fisher's exact test). Testing the frequency of severe dengue between primary and secondary cases also yielded nonsignificant result ($P = 1.0$, two-sided Fisher's exact test). The 95% confidence intervals (CIs) for the percentages are: all cases, dengue infection without warning signs = 6.7–11.2, dengue infection with warning signs = 54.7–62.5, severe dengue infection = 29.1–36.4; primary, dengue infection without warning signs = 6.9–13.2, dengue with warning signs = 52.3–62.7, severe dengue infection = 28.1–38.0; secondary, dengue infection without warning signs = 5.0–11.4, dengue with warning signs = 54.1–65.6, severe dengue infection = 27.1–38.1 (Wilson score interval). **b**, Pie chart showing severe dengue infection, dengue infection with warning signs and dengue infection without warning signs case frequency among children with primary dengue infection who were recruited on or before day 4 after the onset of symptoms and were below detection for dengue-specific IgG using the Panbio Capture ELISA ($n = 126$). The 95% CIs for the percentages are: dengue infection without warning signs = 16.2–29.9, dengue infection with warning signs = 36.6–52.9, severe dengue infection = 25.8–41.3; (Wilson score interval). **c**, Disease severity and incidence of primary and secondary infections as a function of age. The bar graph shows the frequency of primary dengue infections according to age. The number of patients in each age group is indicated in the graph. Infants (1 year old or younger) were all primary infections, which is notably different from the approximately equal mix seen in the older groups ($P \leq 0.00001$, two-sided Fisher's exact test). **d**, Bar graph showing the frequency of severe disease cases in the different age groups. Patients aged 1 year or younger were more likely to have severe dengue compared to older patients ($P \leq 0.002$, two-sided Fisher's exact test). **e**, Pie charts showing the frequency of primary versus secondary infections among severe disease cases in the indicated age groups.

We then determined disease severity in these patients using the WHO 2009 guidelines of dengue infection without warning signs, dengue infection with warning signs and severe dengue infection[2]. Of the 619 children with confirmed dengue who were examined, 202 (32.6%) had severe dengue, 363 (58.6%) exhibited dengue infection with warning signs and 54 (8.7%) had dengue infection without warning signs (Extended Data Table 3). Of importance was the finding that the frequency of severe disease cases was essentially similar in patients with primary (32.5%) versus secondary (32.7%) infections. These data show that disease severity was not preferentially associated with secondary

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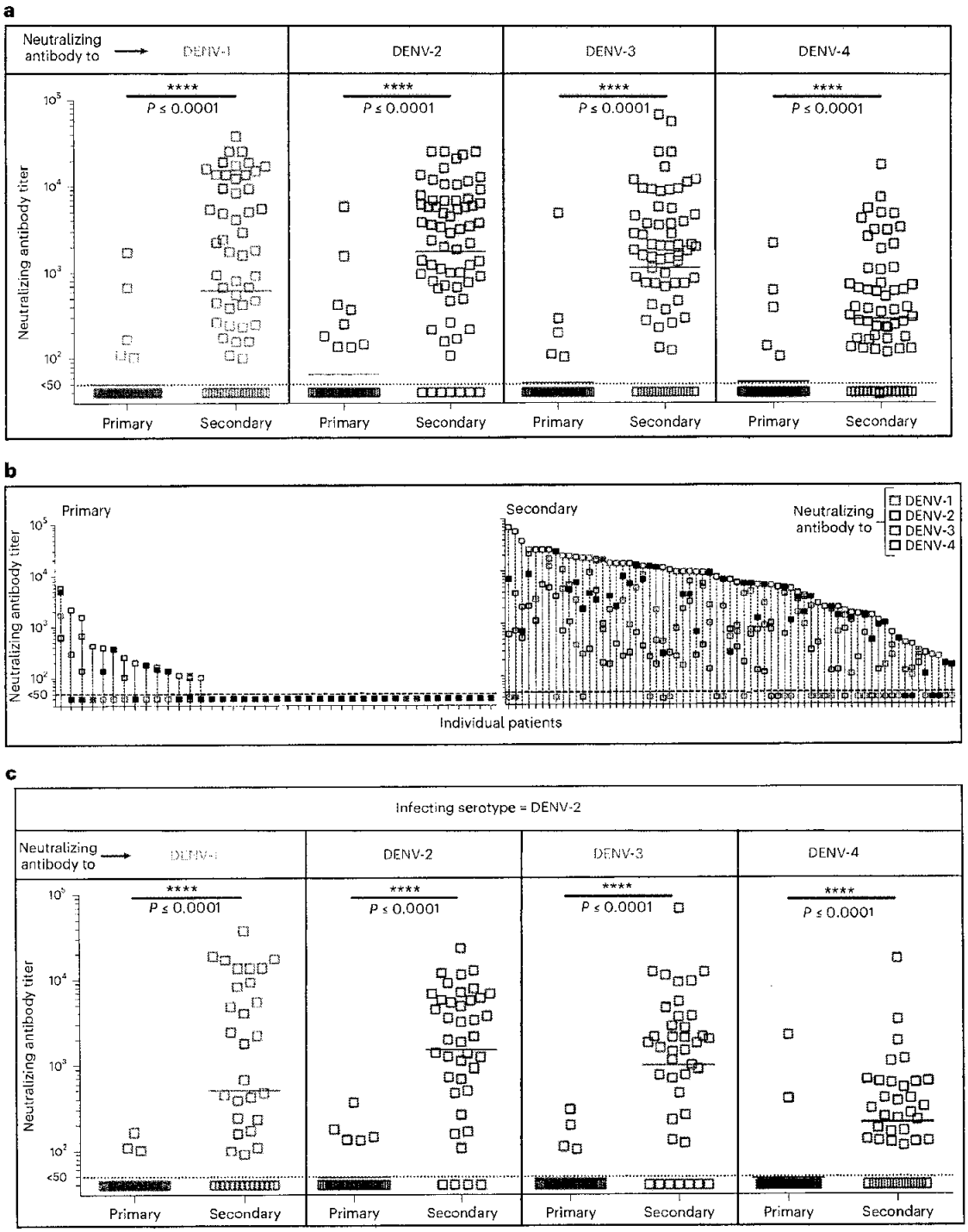

Fig. 2 | Comparison of neutralizing antibody responses between cases with primary and secondary dengue infection. a, Neutralizing antibody titers against each of the four dengue virus serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) in the plasma of a subset of patients from the AIIMS site with primary ($n = 41$) or secondary ($n = 67$) dengue infection. *P* values were calculated using a two-sided Mann–Whitney *U*-test. **b**, Breadth of neutralizing antibody response in individual cases with primary (left) and secondary (right) dengue infection from **a**. Individual patients were stratified from the highest neutralizing titer to any of the four serotypes. The infecting serotype, where known, is indicated by closed symbols. **c**, Neutralizing antibody titers against the infecting virus serotype and heterologous serotypes in primary ($n = 35$) and secondary ($n = 41$) dengue infection from a subset of patients in **a**, where the infecting serotype was DENV-2. *P* values were calculated using a two-sided Mann–Whitney *U*-test.

infections in this study (Fig. 1a). This trend of severe dengue during primary infection was not unique to a single site but was seen at all three clinical site (Extended Data Table 4). Also, severe dengue during both primary and secondary infection was not associated with a particular DENV serotype and was seen with DENV-1, DENV-2 and DENV-3 infections (Extended Data Table 5). There were very few ($n = 8$) DENV-4

infections to make any conclusions about this serotype (Extended Data Table 1).

During these studies, seven fatalities were reported at the AIIMS site (Extended Data Table 6). These patients were all characterized as having severe disease at the time of admission; fatalities occurred within 24–48 h after admission. Five of the seven fatalities were associated with primary dengue infections and two with secondary infections. The IgM:IgG ratios for these patients are shown in Extended Data Table 6.

Our results showing similar frequencies of severe disease in primary and secondary dengue infections was an interesting but surprising finding. To address this issue more rigorously, we increased the classification stringency by using a higher value of IgM:IgG dengue antibody ratio from the WHO-recommended value of 1.2 or higher (as used in Fig. 1a) to 1.32 or 1.4 and 1.78 (Extended Data Fig. 1)[2]. Interestingly, even after increasing the ratio of dengue-specific IgM:IgG antibody to 1.78, the percentage of severe dengue cases in primary infections was comparable (32.5% at the 1.2 ratio versus 33.6% at the 1.78 ratio). Also, the percentage of severe disease cases at the 1.78 cutoff in primary versus secondary infections was highly similar with 33.6% in primary versus 31.9% in secondary infections. As an even more stringent criteria for defining a primary infection, we looked at dengue cases analyzed at early time points after the onset of symptoms (on or before day 4) and were IgG^- at that time for dengue antibody using capture ELISA but IgM^+ for dengue antibody. These IgG^- IgM^+ patients also showed similar frequency (35.7%) of severe disease (Fig. 1b), thus providing compelling evidence of severe disease during primary dengue infection. Also, we further verified that primary infections accounted for a substantial proportion of severe dengue cases by using both the older WHO 1997 disease classification and the newer WHO 2009 classification criteria[2–4] (Extended Data Fig. 2).

We next examined the relative proportions of primary versus secondary dengue infections and disease severity as a function of age. All dengue patients aged 1 year or younger were primary infections whereas the older age groups (years 1–3, 4–6, 7–9, 10–12 and 13 or older) were an equal mix of primary and secondary infections (Fig. 1c). Disease severity was the highest in children aged 1 year or younger compared to older children, where roughly 30% had severe disease (Fig. 1d). It is possible that primary dengue infection might cause severe disease in infants whereas in older children severe disease might predominantly be due to secondary dengue infection. However, this was not the case. Stratification of the data according to age showed that 46.7% of severe disease cases were primary dengue infections in children aged between 1 and 3 years; 50.3% of severe dengue cases were primary dengue infections among children aged 4–16 years (Fig. 1c). Thus, severe dengue was seen during primary dengue infections irrespective of age in these pediatric patients.

It has been proposed that severe dengue in infants is due to the presence of maternal IgG antibodies that cause antibody-dependent enhancement (ADE)[5]. Because more than 60% (22 of 34) of children younger than 1 year had severe disease, we looked at their dengue-specific antibody responses in more detail (Extended Data Fig. 3). Based on the Panbio ELISA, none of these young children ($n = 34$) had any detectable IgG antibody but all were positive for dengue-specific IgM (Extended Data Fig. 3a). Also, neutralizing antibody responses were either low or mostly undetectable in these infants (Extended Data Fig. 3b). We next asked whether IgM levels or neutralizing activity differed in infants with severe dengue infection compared to nonsevere cases. Neither IgM levels (Extended Data Fig. 3c) nor neutralizing antibody responses (Extended Data Fig. 3d) differed significantly depending on disease severity in these infants with primary dengue infection.

To understand if any differences exist in the levels of dengue neutralizing antibody responses during the acute febrile period in children with primary versus secondary dengue infections, we measured the neutralizing antibody titers against each dengue virus serotype (DENV-1, DENV-2, DENV-3 and DENV-4). Samples from children who were categorized as primary infections using capture ELISA showed significantly lower ($P \leq 0.0001$) neutralizing antibody titers for each dengue serotype compared to the samples from children with secondary dengue infection (Fig. 2a). Additionally, samples from children with primary infection (Fig. 2b, left) showed a much narrower breadth of the neutralizing antibody response compared to samples from children with secondary infection (Fig. 2b, right). Analysis of children whose infecting serotype was identified showed that the neutralizing titers to the infecting serotype and for the noninfecting serotypes were below detection or significantly lower in children who were categorized as having primary infections compared to those classified as having secondary infections (Fig. 2c and Extended Data Fig. 4).

It is worth considering our findings in the context of other related studies. Historical studies from Southeast Asia and Cuba attributed the vast majority of dengue hemorrhagic fever/dengue shock syndrome cases to secondary dengue infections[6–8]. However, other studies concluded that primary infections can also cause severe disease and fatalities[9,10]. This interesting and important issue has been much debated; however, during the past several years, many in vitro and animal model studies provided evidence for ADE as a potential mechanism for severe dengue[11,12]. In addition, a more recent study using a cohort in Nicaragua showed enhanced disease in individuals who had an intermediate level of preexisting dengue virus-specific antibody[13]. Thus, the prevailing consensus in the field is that severe dengue is tightly linked to secondary dengue infections. Our study of pediatric dengue infections in India shows that severe disease is also seen in primary infections. The results of our study do not necessarily question ADE or the presentation of severe disease during secondary dengue; rather, they highlight that primary infections can also be a major contributor to the dengue disease burden.

It is important to discuss some of the limitations of our study. The Panbio capture ELISA assay we used in this study is standardized and widely used all over the world for classifying primary versus secondary dengue infections; however, it is not the most sensitive assay[14–16]. Thus, we cannot rule out potential misclassification in some of the primary cases. Also, we cannot make any definitive statements about maternal antibodies using this Panbio assay. Finally, our study was conducted in tertiary clinical centers and may not necessarily predict what is happening at the population level in the community[17]. Future studies using well-designed cohort-based studies are needed to address this important question. We hope that the interesting results from our study of more than 600 patients from tertiary care hospitals in three different geographical areas of India will provide the impetus for carrying out larger community-level, population-based studies in India.

Our studies are relevant to the ongoing global dengue vaccine development efforts that are strongly influenced by the view that severe dengue is overwhelmingly seen during secondary infection[18–20]. Hence, dengue vaccine trials are often done in seropositive individuals to boost their immunity; recently approved dengue vaccines are mostly licensed for seropositive individuals[18]. Our results show that primary dengue infections can also constitute a substantial fraction of the burden of severe disease. Thus, there is an urgent need for an effective dengue vaccine that can be safely used in dengue-naive individuals.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-024-02798-x.

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Methods

Study oversight and sample acquisition

Our research complies with all relevant ethical regulations and was approved by Institutional Ethics Committees of AIIMS, SJRI and CMC, where the patients were recruited. A total of 619 cases with confirmed dengue infection in children younger than 17 years recruited from three different tertiary clinical sites located in diverse geographical regions of India are included in this analysis. This included the SJRI ($n = 380$, during 2014–2016) located in Southern India, the AIIMS ($n = 200$, during 2012–2018) located in Northern India, and CMC ($n = 39$, during 2015–2017) located in Southeastern India. Written informed consent was obtained from the parent or guardian of the child; verbal assent was obtained from children aged 8 years and older. Participation in the study was voluntary. Children eligible for enrollment at the SJRI site were those admitted to the inpatient pediatric unit with a clinical diagnosis of dengue, which was made by the treating physician using the WHO 2009 guidelines. Eligible children at the AIIMS and CMC sites were those admitted to the inpatient pediatric unit with a clinical diagnosis of dengue and those reporting to the outpatient center with mild dengue as determined by a combination of clinical diagnosis and a positive rapid test for dengue nonstructural protein 1 (NS1)/IgM or IgM ELISA. Cases positive for malaria were excluded. No previous medical records or information was available regarding a history of past confirmed or suspected dengue infections. Inclusion criteria consisted of being aged from 1 month to 17 years at the SJRI site, or 4–14 years at the AIIMS and CMC sites, a clinical diagnosis of dengue, written informed consent by the parent or guardian of the child, and verbal assent from children aged 8 years and older. Blood samples obtained to perform routine hospital screening were evaluated for stringent laboratory confirmation of recent dengue virus infection and negativity for chikungunya IgM and malaria antigens as outlined in the following sections. A total of 619 patients, consisting of 355 males and 264 females, with confirmed dengue virus infection and negative for chikungunya IgM and malaria antigen were included in this analysis. Individualized de-identified data on age, sex and clinical disease classification of the patients analyzed in this study are provided as supplementary source data. All raw data are provided as source files or presented in the main text or extended data.

Disease characterization

Based on extensive clinical laboratory tests and evaluation, the attending physicians at each clinical site classified the dengue disease grade based on the WHO 2009 guidelines as dengue infection without warning signs (DI), dengue infection with warning signs (DW) and severe dengue infection (SD)[2] at the time of recruitment. The disease grade compiled by the attending physician was not disclosed to the researchers until the end of the study, allowing for a blinded study.

Plasma isolation

Blood samples were collected in Vacutainer CPT tubes (catalog no. 362761, BD Biosciences). Plasma was separated as described in previous studies[21].

Dengue confirmation

Laboratory confirmation of dengue infection was based on positivity to one or more of the following: dengue NS1 capture ELISA (catalog no. IR31048, J Mitra), dengue IgM Capture ELISA (catalog no. 01PE20, Panbio) and dengue real-time PCR. Only patients who were positive for dengue NS1 or IgM or PCR, and negative for chikungunya IgM strip test (catalog no. IR061010, J Mitra) and negative for malaria antigen ELISA (catalog no. 05EK40, SD Bioline), were included in this analysis.

Characterization of primary and secondary infections

Primary and secondary infections were classified using standard Panbio Capture ELISAs (catalog nos. 01PE10 and 01PE20) that quantify IgM and IgG ratios in plasma diluted 1:100. In patients who were seronegative in the first sampling, a second febrile bleed was used to classify primary versus secondary status. Unless otherwise mentioned, and according to the WHO criteria, within confirmed dengue patients, primary dengue infections were those that did not induce detectable levels of antibodies in the subsequent two samples or had an IgM:IgG ratio of 1.2 or higher and secondary infections were those that had an IgM:IgG ratio of less than 1.2 (ref. 2).

Dengue virus neutralization using focus-forming assay

The neutralization capacity of plasma samples against each of the dengue virus serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) was determined using a focus-forming assay as described previously[22]. Plasma was heat-inactivated at 56 °C for 30 min. Serially diluted plasma (1:100–1:102,400) was incubated with 100 focus-forming units of virus for 1 h at 37 °C. Vero cell monolayers were infected with the plasma–virus mixture for 1 h at 37 °C. An overlay containing 2% carboxymethylcellulose was added to the infected cells. After 72 h, cells were fixed and then stained with anti-flavivirus monoclonal antibody 4G2 (catalog no. MAB10216, Merck Millipore) followed by detection with horseradish peroxidase-linked anti-mouse IgG (catalog no. 7076S, Cell Signaling Technology). Foci were developed using TrueBlue Peroxidase (catalog no. 50-78-02, KPL) as a substrate. Neutralizing antibody titers were calculated as the reciprocal of the plasma dilution where 50% reduction in foci was observed. Samples in which at least a 50% reduction in foci was not seen at 1:50 dilution were considered below the assay cutoff and were assigned a value of 1:40.

Serotype determination

Viral RNA was extracted from 120 µl whole blood and the dengue virus serotype was detected based on previously published protocols[23].

Statistical analysis

Data were analyzed using R v.4.3.1 and Prism 6 (GraphPad Software). $P < 0.05$ was considered statistically significant.

Ethics and inclusions

Clinical data pertaining to this study were collected in India. Thirty colleagues, including the senior authors (R.L., A.S. and AC.), are from India. We fully endorse and are committed to the guidance of the Nature Portfolio journals on low- and middle-income country authorship and inclusion.

Our research complies with all relevant ethical regulations and was approved by Institutional Ethics Committees of AIIMS, SJRI and CMC, where patients were recruited. Study participation was voluntary. The data collection and analysis techniques used raised no risks pertaining to incrimination, discrimination, the environment, health, safety, security or other personal risks. No cultural artifacts or associated traditional knowledge has been transferred out of any country. This research is locally relevant to India and globally.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All the raw data analyzed are provided as source files in the main text and in the extended data material. Individual de-identified data for age, sex and clinical disease classification are provided as source data in the supplementary information. Source data are provided with this paper.

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23. Kar, M. et al. Isolation and molecular characterization of dengue virus clinical isolates from pediatric patients in New Delhi. *Int. J. Infect. Dis.* **84S**, S25–S33 (2019).

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Author contributions

M.S., S.F.A., R.V., S.M., A.R., V.P.V., A.M.A., S.K.K., R.L. and A.S. carried out patient recruitment and follow-up. C.A., H.A., P. Sharma, H.P., K.N., R.C.R., D.M., S.G., L.P., S.K.B., S.F.A., R.V., E.S.R., Y.M.C., P. Bhatnagar, P. Singh, M.K., K.D., S.K., K.G., K.S., P. Bajpai, G.P.S., P. Shah, A.K., T.Y., C.W.D., R. Antia and G.R.M. performed the experiments, analysis and interpretation. J.W., A.A., A.M.A., S.K.K., R. Ahmed, R.L., A.S., A.C. and K.M-K. were involved in study design, analysis and interpretation. C.A., H.A., R. Ahmed, R.L., A.S., A.C. and K.M-K. prepared the paper.

Competing interests

The authors declare no competing interests.

Additional information

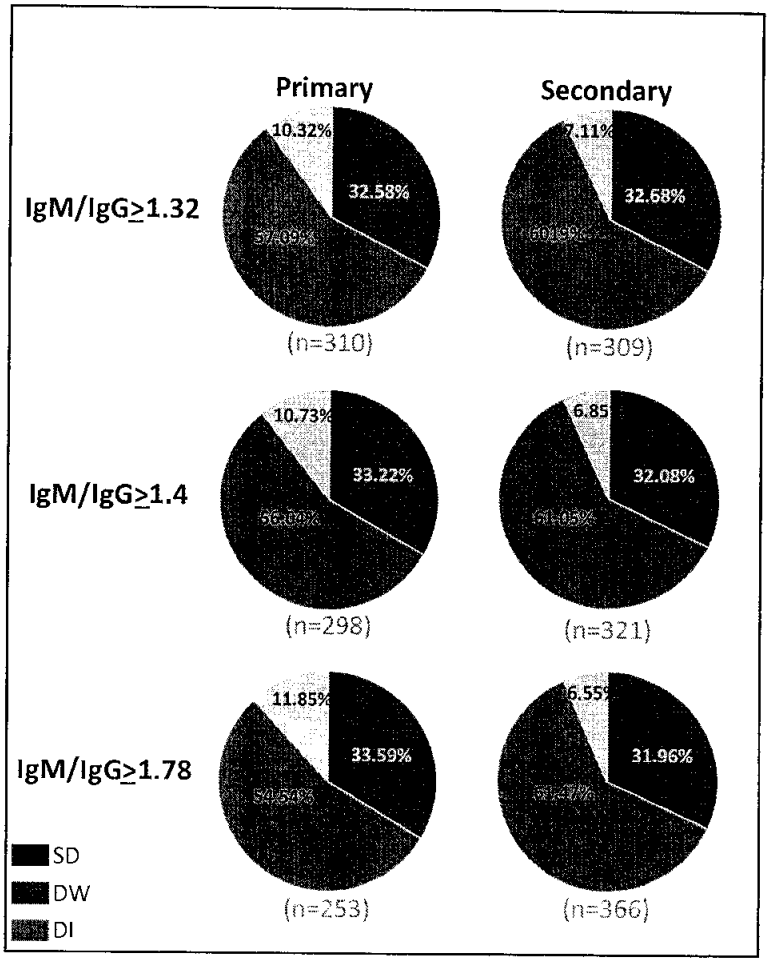
Extended data is available for this paper at https://doi.org/10.1038/s41591-024-02798-x.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-024-02798-x.

Correspondence and requests for materials should be addressed to Rafi Ahmed, Rakesh Lodha, Anita Shet, Anmol Chandele or Kaja Murali-Krishna.

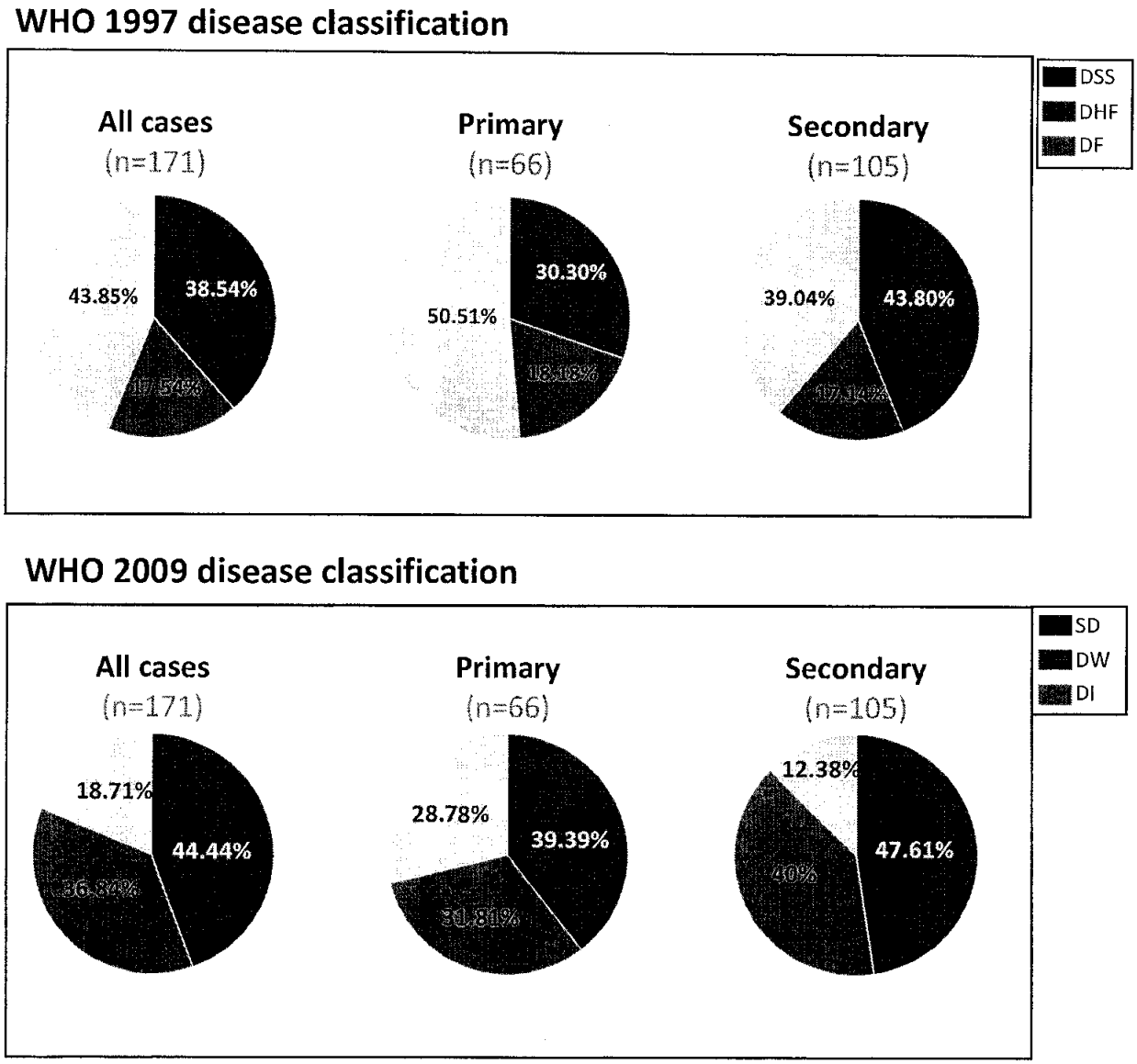
Peer review information *Nature Medicine* thanks Eng Eong Ooi and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: Saheli Sadanand, in collaboration with the *Nature Medicine* team.

https://doi.org/10.1038/s41591-024-02798-x



Extended Data Fig. 1 | Similar frequency of severe disease in primary versus secondary cases that were distinguished using stringent IgM/IgG ratios. Pie charts show the frequency of Severe Dengue (SD), Dengue with warning signs (DW) and Dengue infection without warning signs (DI) cases in primary versus secondary dengue infections that were distinguished using more stringent IgM/IgG ratios indicated on left. The number of patients in each group is indicated below the pie chart. For all three classification methods, the proportion of severe disease was not significantly different between primary and secondary cases ($p > 0.78$, two-sided Fisher's exact test). The 95% confidence interval for the percentages indicated in the pie charts are as below: IgM/IgG >1.32, primary: DI-5.4-11.6, DW-53.4-64.4, SD-27.9-38.5, Secondary: DI-6.7-13.1, DW-52.8-63.6, SD-27.4-37.6; IgM/IgG >1.4: primary: DI-5.7-12.1, DW-52.2-63.5, SD-28.5-39.3, secondary: DI-6.4-12.6, DW-53.8-64.4, SD-26.9-36.9; IgM/IgG >1.78: primary: DI-5.8-13.0, DW-50.5-62.9, SD-28.7-40.6 and secondary: DI-6.3-12.0, DW-54.8-64.6, SD-27.0-36.3 (Wilson CI).

https://doi.org/10.1038/s41591-024-02798-x



Extended Data Fig. 2 | Frequency of severe disease in primary versus secondary dengue infections using WHO 1997 and WHO 2009 disease classification. Data from a subset of the patients from the AIIMS Delhi site where disease severity was classified using both WHO 2009 and WHO 1997 criteria. **a**, Data shown by WHO 1997 disease classification. Pie charts show the frequency of the cases with dengue shock syndrome (DSS), dengue hemorrhagic fever (DHF); or dengue fever (DF) among a subset of dengue confirmed children that are recruited from AIIMS site among all cases (n = 171), primary dengue cases (n = 66) and secondary dengue cases (n = 105). DSS case frequency is not significantly different between the primary and secondary dengue infections, ($p = 0.106$, two-sided Fisher's exact test). **b**, Data shown by WHO 2009 disease classification among the same group of the patients from panel a. Pie charts show the frequency of the cases with severe dengue (SD), dengue with warning signs (DW); or dengue infection without warning signs (DI) among all cases, primary dengue cases or secondary dengue cases. Severe dengue case frequency was not significantly different between the primary and secondary dengue infections, ($p = 0.344$, two-sided Fisher's exact test).

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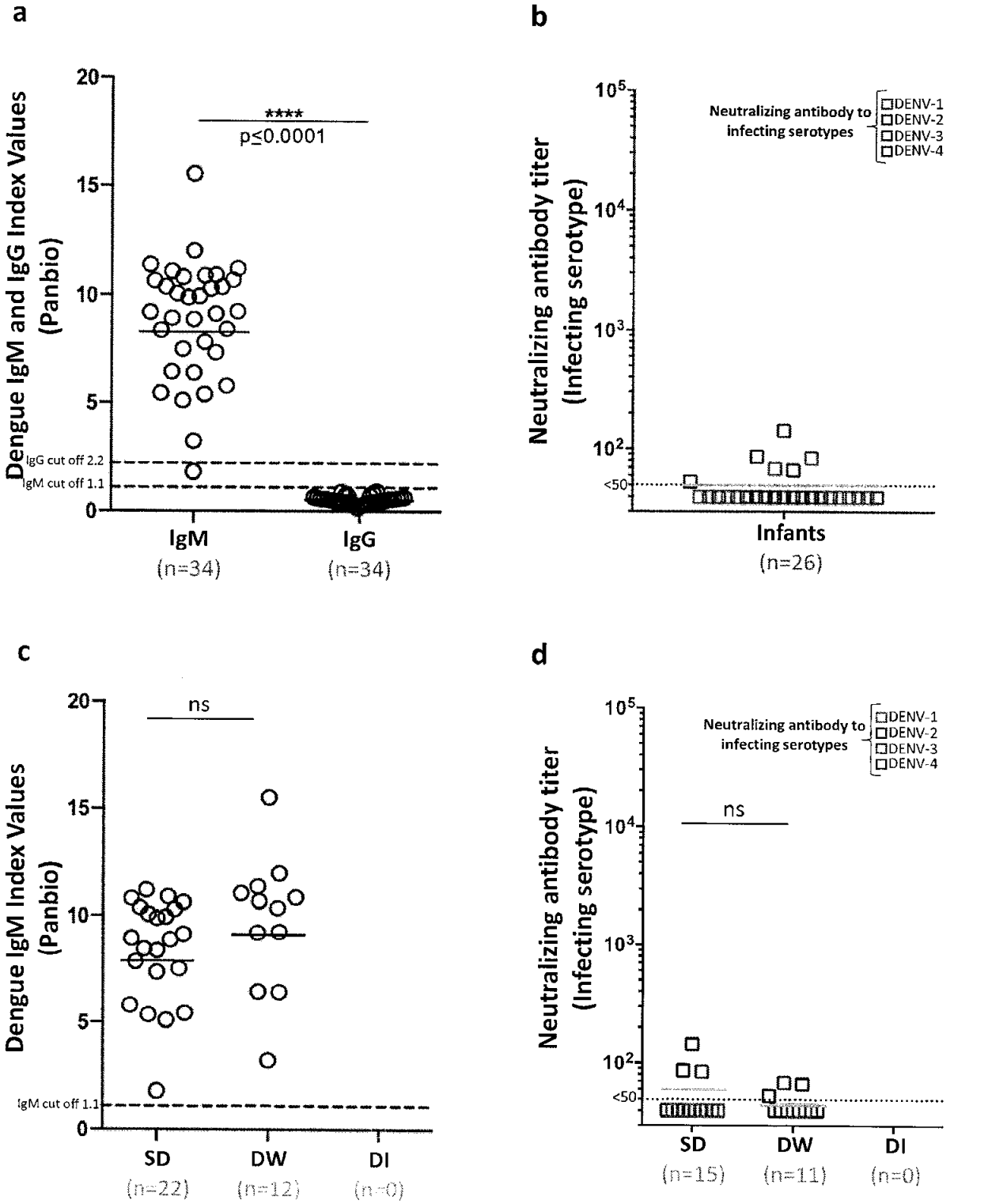
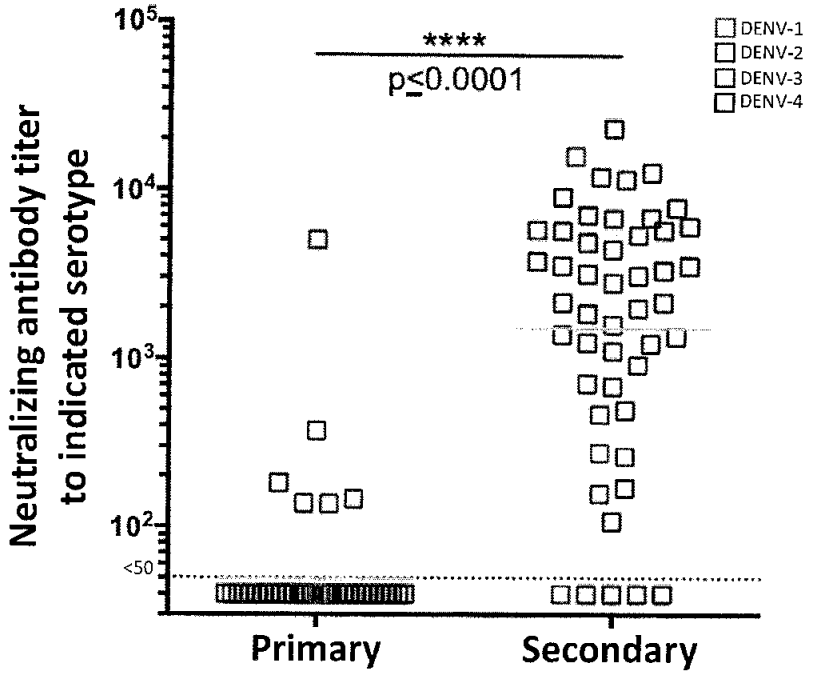


Extended Data Fig. 3 | Dengue specific responses in infants (≤1-year-old). **a**, Scatter plot shows dengue specific IgM and IgG index values by capture Elisa (Panbio) for dengue confirmed infants (n = 34). p values were calculated using two-sided Mann-Whitney U tests **b**, Neutralizing antibody titers to the indicated infecting virus serotype in dengue confirmed infants where the infecting serotype was determined (n = 26). **c**. Scatter plots show dengue specific IgM index values by Panbio Capture ELISA among the infants with different grades of disease severity. Severe dengue (SD, n = 22); Dengue with warning signs (DW, n = 12). Note that there are no Dengue infection without warning signs (DI) cases since all the hospitalized infants were either SD or DW cases. p values (p = 0.087) were calculated using two-sided Mann-Whitney U tests. Non-significant p values (>0.05) are indicated as n.s. **d.** Scatter plots show neutralizing activity against the indicated infecting dengue virus serotypes among the infants with different grades of disease severity. Severe dengue (SD, n = 15); Dengue with warning signs (DW, n = 11). Note that there are no DI cases since all of the hospitalized infants were either SD or DW cases. p values (p > 0.999) were calculated using two-sided Mann-Whitney U tests. Non-significant p values (>0.05) are indicated as n.s.

https://doi.org/10.1038/s41591-024-02798-x



Extended Data Fig. 4 | Neutralization responses were below detection or significantly lower for infecting serotype in the primary dengue cases compared to secondary dengue cases. Neutralizing antibody titers against the infecting virus serotype in primary (n = 38) and secondary (n = 50) from a subset of the patients from 2b, where the infecting serotype was identified. p values were calculated using Mann-Whitney U test.

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Extended Data Table 1 | Characteristics of dengue-infected patients

Characteristics of the dengue infected patients (n=619).

	All Sites Pooled (n=619)	Clinical Sites		
		SJRI[a] Bengaluru (n=380)	AIIMS[b] Delhi (n=200)	CMC[c] Vellore (n=39)
Male/Female (n)	355/264	227/153	105/95	23/16
Age in years, average (Range)	7.9 (0.2-16.1)	7.1 (0.2–16.1)	9.3 (4-14)	8.7 (4-14)
Day of symptoms, average (Range)	4.4 (2-13)	4.3 (2–7)	4.4 (2–7)	6.6 (3-13)
Serotype determined, n	411	257	154	
DENV-1 , n (%)	188 (45.7%)	183 (71.2%)	5 (3.2%)	
DENV-2, n (%)	143 (34.7%)	48 (18.6%)	95 (61.7%)	
DENV-3, n (%)	55 (13.4%)	12 (4.6%)	43 (27.9%)	
DENV-4, n (%)	8 (1.9%)	2 (0.8%)	6 (3.9%)	
>1 serotype, n (%)	17 (4.1%)	12 (4.7%)	5 (3.2%)	
Serotype not determined, n	208	123	46	39

[a]*SJRI- St. John's Research Institute.*
[b]*AIIMS- All India Institute of Medical Sciences.*
[c]*CMC- Christian Medical College.*

https://doi.org/10.1038/s41591-024-02798-x

Extended Data Table 2 | Primary versus secondary infection status of patients with confirmed dengue infection

Primary versus secondary infection status of the dengue confirmed patients.

Clinical site*	Primary n (%)	Secondary n (%)
All sites (n=619)	344 (55.6%)	275 (44.4%)
SJRI, Bengaluru (n=380)	239 (62.9%)	141 (37.1%)
AIIMS, Delhi (n=200)	86 (43%)	114 (57%)
CMC, Vellore (n=39)	19 (48.7%)	20 (51.2%)

**SJRI- St. John's Research Institute.*
AIIMS- All India Institute of Medical Sciences.
CMC- Christian Medical College.

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Extended Data Table 3 | Disease characteristics of patients with confirmed dengue infection

Disease characteristics of the dengue confirmed patients (n=619).

	All Sites Pooled (n=619)	Clinical Site		
		SJRI[a] Bengaluru (n=380)	AIIMS[b] Delhi (n=200)	CMC[c] Vellore (n=39)
Severe Dengue (SD)*	202 (32.6%)	114 (30.0%)	83 (41.5%)	5 (12.8%)
Dengue Warning (DW)*	363 (58.6%)	262 (68.9%)	73 (36.5%)	28 (71.8%)
Dengue Infection (DI)*	54 (8.7%)	4 (1.1%)	44 (22.0%)	6 (15.3%)

**based on WHO 2009 disease severity classification.*

[a]*SJRI- St. John's Research Institute.*
[b]*AIIMS- All India Institute of Medical Sciences.*
[c]*CMC- Christian Medical College.*

https://doi.org/10.1038/s41591-024-02798-x

Extended Data Table 4 | Disease severity between primary and secondary dengue infection at individual clinical sites

Disease severity among primary versus secondary dengue infections at individual clinical sites.

Clinical Site*	Primary/ Secondary	Disease severity		
		Severe Dengue n (%)	Dengue Warning n (%)	Dengue Infection n (%)
SJRI, Bengaluru	Primary (n=239)	77 (32.2%)	160 (66.94%)	2 (0.83%)
	Secondary (n=141)	37 (26.2%)	102 (72.3%)	2 (1.41%)
AIIMS, Delhi	Primary (n=86)	31 (36.04%)	27 (31.39%)	28 (32.55%)
	Secondary (n=114)	52 (45.61%)	46 (40.35%)	16 (14.03%)
CMC, Vellore	Primary (n=19)	4 (21.05%)	11 (57.89%)	4 (21.05%)
	Secondary (n=20)	1 (5%)	17 (85%)	2 (10%)

**SJRI- St. John's Research Institute.*
AIIMS- All India Institute of Medical Sciences.
CMC- Christian Medical College.

https://doi.org/10.1038/s41591-024-02798-x

Extended Data Table 5 | Disease severity between patients with primary and secondary dengue infection depending on dengue serotype

Disease severity among primary versus secondary dengue infected patients depending on the dengue serotype.

Serotype*	Primary/ Secondary	Disease severity, n(%)		
		Severe Dengue	Dengue Warning	Dengue Infection
DENV-1	Primary (n=143, 76.1%)	47 (32.9%)	93 (65.0%)	3 (2.1%)
	Secondary (n=45, 23.9%)	14 (31.1%)	31 (68.9%)	0 (0%)
DENV-2	Primary (n=75, 52.4%)	26 (34.7%)	33 (44.0%)	16 (21.3%)
	Secondary (n=68, 47.6%)	33 (48.5%)	30 (44.1%)	5 (7.4%)
DENV-3	Primary (n=26, 47.3%)	12 (46.2%)	9 (34.6%)	5 (19.2%)
	Secondary (n=29, 52.7%)	12 (41.4%)	14 (48.3%)	3 (10.3%)

* *From PCR confirmed cases.*

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Extended Data Table 6 | Fatalities in primary and secondary dengue infections

Fatalities in primary and secondary dengue infections.

Patient	Primary/ Secondary	Age (years)	Infecting Serotype	Day of fatality post onset of symptoms*	Panbio Capture ELISA		
					IgM**	IgG***	IgM/ IgG$
Patient 1	Primary	9	DENV-2	6	0.31	0.12	-
Patient 2	Primary	9	DENV-3	5	6.65	2.74	2.42
Patient 3	Primary	8	DENV-2	4	1.15	0.19	6.05
Patient 4	Primary	5	DENV-2	4	0.52	0.25	-
Patient 5	Primary	5	DENV-2	4	9.76	0.28	34.85
Patient 6	Secondary	5	DENV-3	4	3.75	6.80	0.55
Patient 7	Secondary	4	DENV-2	7	1.1	2.47	0.44

* *All samples are from AIIMS, New Delhi. All samples were classified as severe disease (SD) at the time af admission.*
** *By PanBio capture ELISA, assay cut off 1.1*
*** *By PanBio capture ELISA, assay cut off 2.2*
$ *Ratios were not calculated when IgM and IgG index values were below assay cut off.*

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Last updated by author(s): 12/20/2023

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
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☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection: No software was used for data collection.

Data analysis: Data was analyzed using R. 4.3.1 and GraphPad Prism.

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- Accession codes, unique identifiers, or web links for publicly available datasets
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- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All the raw data analyzed are provided as source files in the main text and in the extended data material. Individual de-identified data for age, sex, and clinical disease classification are provided as source data in the supplementary information.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	This study includes both males and females sexes based on self-reporting or reporting by the parent/guardian. Overall numbers studied include 355 males and 264 females.
Population characteristics	A total of 619 cases of confirmed dengue infection in children of age less than 17 years recruited from three different tertiary clinical sites located in diverse geographical regions of India are included in this analysis. This included the St. John's Research Institute (SJRI), Bengaluru (n=380, during the years 2014-2016) located in Southern India; All India Institute of Medical Sciences (AIIMS), New Delhi, (n=200, during the years 2012-2018) located in Northern India; and Christian Medical College (CMC), Vellore, (n=39, during the years 2015-2017) located in Southeastern India.
Recruitment	Written informed consent was obtained from parent/ guardian of the child, and verbal assent was obtained from children 8 years and older. Participation in the study was voluntary. Eligible children for enrollment at the SJRI site were those admitted to inpatient pediatric unit with clinical diagnosis of dengue, that was made by the treating physician using the WHO 2009 guidelines. Eligible children at the AIIMS and CMC sites were those admitted to inpatient pediatric unit with clinical diagnosis of dengue as well as those reporting to outpatient center with mild dengue as determined by a combination of clinical diagnosis and a positive rapid test for dengue NS1/IgM and or IgM ELISA. Malaria positive cases are excluded. No prior medical records or information was available regarding history of past confirmed or suspected dengue infections. Inclusion criteria consisted of ages 1 month to 17 years (at SJRI site) or ages 4-14 years (at the AIIMS and CMC sites), a clinical diagnosis of dengue, a written informed consent by the parent/ guardian of the child and a verbal assent from children 8 years and older. Blood samples that were obtained to perform routine hospital screening were evaluated for stringent laboratory confirmation of recent dengue virus infection and negativity for chikungunya IgM/ malaria antigens as outlined in sections below. A total of 619 patients, comprising 365 males and 264 females, that were confirmed as dengue virus infection and negative for chikungunya IgM and malaria antigen are included in this analysis.
Ethics oversight	Our research complies with all relevant ethical regulations and was approved by Institutional Ethical Committees of the All India Institute of Medical Sciences (AIIMS, New Delhi, India), St. John's Research Institute (SJRI, Bengaluru, India), Christian Medical College (CMC, Vellore, India), where the patients were recruited.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample size was not predetermined prior to the study. This is a cross-sectional, observational study. The study included dengue confirmed, chikungunya and malaria negative febrile children from St. John's Research Institute (SJRI), Bengaluru (n=380, during the years 2014-2016) located in Southern India; All India Institute of Medical Sciences (AIIMS), New Delhi, (n=200, during the years 2012-2018) located in Northern India; and Christian Medical College (CMC), Vellore, (n=39, during the years 2015-2017) located in Southeastern India. Written informed consent was obtained from parent/ guardian of the child, and verbal assent was obtained from children 8 years and older. Participation in the study was voluntary.
Data exclusions	Analysis presented here was performed on the available data from 619 individuals. No data was excluded from the analysis.
Replication	The study design did not require replicates. A total of 619 patients were analyzed and were utilized for statistical calculations and inferences made in the study.
Randomization	This is not a randomized study.
Blinding	Blinding was not relevant to this study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used: NS1 capture ELISA (J Mitra, Cat# IR31048), Dengue IgM Capture ELISA (Pan bio, Cat# 01PE20), Chikungunya IgM strip test (J Mitra, Cat# IR061010) Malaria antigen ELISA (SD Bio line, Cat# 0SEK40). PanBio capture ELISA for dengue IgM and IgG (Cat# 01PE10/01PE20). Anti-pan flavivirus monoclonal antibody (4G2 (Millipore; Cat# MAB10216)); HRP-linked anti-mouse IgG (Cell Signaling; Cat# 7076S). All antibodies were used at the dilutions recommended by the manufacturers.

Validation: Commerical ELISA assays were performed as per the manufacturer's instructions and thus did not require further validation. All antibodies were used at the dilutions recommended by the manufacturers.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): ATCC Vero cell line - CCL 81 was used for dengue neutralization assays.

Authentication: Not authenticated.

Mycoplasma contamination: Vero cell lines were mycoplasma negative. Mycoplasma detection PCR was performed using Thermo scientific PCR mycoplasma detection kit (J66117.AMJ).

Commonly misidentified lines (See ICLAC register): None.