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Reporting Summary

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Statistics

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
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ 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	N/A
Data analysis	All statistical analyses were performed using R (v4.2.3, R Foundation for Statistical Computing). Differences in the ED50 of binding antibodies in pre-infection samples between children who subsequently experienced dengue fever (DF) or dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) were analyzed using a two-tailed Wilcoxon rank-sum test with false discovery rate (FDR) correction for multiple comparisons. Logistic regression models were employed to evaluate the association between pre-existing antibody profiles and the subsequent development of DHF/DSS. Each antibody characteristic was analyzed in a separate regression model, with FDR correction applied for multiple hypothesis testing. To adjust the coefficients of the antibody characteristics correlated with DHF/DSS, we performed a multivariate regression with regularization. To estimate the regularization parameters, the dataset was divided into training and testing sets (50-50 split). Optimal regularization parameters (gamma and alpha) were determined via bootstrapping (n=1000), maximizing the area under the curve (AUC) on the training set. Then, the optimal parameters were applied to re-estimate the coefficients for selected antibody features using the entire dataset. Regularization was implemented using the glmnet R package (v4.1-860). To compare the predictive accuracy of Total IgA versus Total IgG antibody avidity in pre-infection samples for distinguishing the children who subsequently developed DF or DHF/DSS, a similar regularization approach was used. In addition, the variables in the final model were assessed using an orthogonal partial least squares discriminant analysis (OPLS-DA) to estimate the variance in the response variable explained by the model (R^2Y), the predictive performance for new data (Q^2), and the overall classification accuracy. Three models were compared: avidity of IgA antibodies alone, avidity of IgG antibodies alone, and a model including avidity of IgA + IgG.

Normalized phagocytic scores, percentage changes in signal in the NETosis assay, relative neutrophil counts, and levels of degranulation markers were compared across clinical groups using a one-tailed Wilcoxon rank-sum test. Logistic regression was employed to assess the association between degranulation marker levels and severe disease manifestations, estimating odds ratios (ORs) and 95% confidence intervals (CIs) using the oddsratio package (v2.0.1). Linear regression was used to evaluate the relationships between degranulation markers and antibody binding profiles, as well as between degranulation markers and maximum gallbladder wall thickness. All statistical analyses were conducted at a significance level of 5%. Reference code and datasets for antibody profiling and coefficient adjustment using regularization (DOI: 10.5281/zenodo.14837883), reference code for assessing the classification performance of IgA, IgG, and IgG/IgA avidity (DOI: 10.5281/zenodo.14837889), and source data for figures (DOI: 10.5281/zenodo.14837915) are available for reference on Zenodo.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data associated with this study are presented in the main text or supplementary materials. The associated code is available for reference on Zenodo, including reference code and datasets for antibody profiling and coefficient adjustment using regularization (DOI: 10.5281/zenodo.14837883), reference code for assessing the classification performance of IgA, IgG, and IgG/IgA avidity (DOI: 10.5281/zenodo.14837889), and source data for figures (DOI: 10.5281/zenodo.14837915). After securing approval from the UC Berkeley Committee for the Protection of Human Subjects, individual data for figure reproduction can be shared with external researchers. For data access arrangements, please contact E.H. at eharris@berkeley.edu. Standard data and material transfer agreements govern all data used in this study.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Information about sex was collected based on self-reporting. Participant characteristics are summarized in Table S1.
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	Participant characteristics are summarized in Table S1.
Recruitment	The Pediatric Dengue Cohort Study (PDCS) is an ongoing prospective cohort study initiated in 2004 based at the Sócrates Flores Vivas Health Center (HCSFV), the primary health care facility for District II of Managua and part of the Municipal Health System of Managua. Located adjacent to Lake Xoxitlan, District II encompasses neighborhoods spanning a spectrum of low to middle socioeconomic status. The HCSFV serves ~62,000 residents across 18 neighborhoods within District II. The illiteracy rate in this area is approximately 7%, and over 90% of the households have access to running water and a sewage system. All residents have access to electricity. The PDCS tracks approximately 4,000 active participants ages 2-17 yearly and has followed ~12,000 children since its initiation. Initial enrollment in 2004 was children 2-9 years old; adjustments to the age limit were implemented in 2008, 2013, 2018, 2019, and 2022, extending the eligibility to 11, 14, 15, 16, and 17 years, respectively. To preserve the age distribution of the cohort, approximately 250 two-year-old children, along with children aged 3-11 years as needed, are enrolled annually. Participants benefit from free, round-the-clock 24/7 medical care provided by study physicians at the HCSFV. In cases requiring hospitalization, study staff facilitate patient transfer to the study hospital, the National Pediatric Reference Hospital (Hospital Infantil Manuel de Jesús Rivera). Children presenting with fever and, since July 2016, rash are screened for signs and symptoms of dengue, Zika, and chikungunya. Symptomatic infections are captured through “enhanced” passive surveillance, complemented with periodic home visits. For each suspected case, both acute (0-6 days post-symptom onset) and early convalescent samples (14-21 days) are collected to confirm infection. Approximately 97% of cases present to the HCSFV within the first three days of illness, and convalescent samples are collected from 94% of cases (Kuan et al 2009 AJE); similar statistics have been maintained to date. Additionally, the study collects annual healthy blood samples from all participants in March-April (previously in July from 2004-2010), which enables the detection of inapparent arboviral infections. The Pediatric Dengue Hospital-Based Study (PDHS) is based at the Hospital Infantil Manuel de Jesús Rivera (HIMJR), Managua, Nicaragua (2005-present). Children are invited to participate if they: 1) present to the hospital 1-7 days post-onset of symptoms and 2) meet case definitions for dengue, Zika, and/or chikungunya, which consist of either i) ≥1 signs or symptoms, including headache, arthralgia, myalgia, retro-orbital pain, positive tourniquet test, petechiae, or signs of bleeding; or ii) rash with ≥2 signs/symptoms including fever, conjunctivitis, arthralgia, myalgia, or peri-articular edema. Blood samples are collected at presentation (acute), daily for days 1-3, and at early convalescence (14-28 days). Clinical information (>150 variables) and fluid intake/output is collected every 12 hours, and, after systematic monitoring by a clinical supervisor, is digitized by double-data entry, with quality control checks performed daily and weekly. A standardized questionnaire is used to record the subject’s demographic and epidemiologic characteristics. As part of a longitudinal arm of the study, consenting participants return to provide healthy blood samples 3, 6, 12, and 18 months post-illness. For both studies, infections are defined using real-time RT-PCR, virus isolation, and DENV, ZIKV, and CHIKV serological assays.

Ethics oversight	The Pediatric Dengue Cohort Study was reviewed and approved by the institutional review boards of the University of California, Berkeley (protocol 2010-09-2245), the University of Michigan (HUM00091606), and the Nicaraguan Ministry of Health (NIC-MINSA/CNDR CIRE-09/03/07-008). The Pediatric Dengue Hospital-based Study was reviewed and approved by the institutional review boards of the University of California, Berkeley (2010-06-1649) and the Nicaraguan Ministry of Health (NIC-MINSA/CNDR CIRE-01/10/06-13). Parents or legal guardians of all subjects provide written informed consent, and subjects ≥6 years old provide assent.

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

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	All participants from the PDCS who had a documented primary ZIKV infection in 2016 followed by a second DENV2 infection during 2019-2020, with the second infection classified as DHF/DSS according to WHO 1997 criteria, were included (n=10). Additionally, 14 participants with a similar infection history who developed DF, matched by sex and age, were selected as a comparison group using convenience sampling, as well as 17 participants with the same infections history who developed a second inapparent DENV2 infection. The symptomatic sample set (DF vs. DHF/DSS) was used for analysis of neutrophil counts during infection, excluding 7 participants with missing neutrophil counts on day 3 post-fever onset (Fig. 3B). In the PDHS, a total of 362 confirmed DENV2 infections were documented between 2018 and 2020. Among these, 261 cases were classified as DF and 65 as DHF/DSS. All 65 DHF/DSS and a convenience sample of 103 DF cases were serotyped to identify second DENV2 infections after a primary ZIKV infection, using the methods described in references 4 and 12. A total of 111 participants with past ZIKV infections were identified (DF=67, DHF/DSS=44). This entire sample set was used for analysis of neutrophil counts during infection, excluding 2 participants with missing neutrophil counts (Fig. 4A). For our orthogonal confirmation of neutrophil involvement during acute DENV2 infection, we determined the sample size using Cohen's d effect size and power analysis. Based on our observations in the PDCS, the estimated effect size was d = -0.9128 (95% CI: -1.8139, -0.0117), corresponding to a large effect size. To achieve a statistical power of 80% ($\beta = 0.20$) at a significance level of 0.05 ($\alpha = 0.05$) for a one-tailed two-sample t-test (assuming normal data distribution), a minimum of 16 participants per group (DF vs. DHF/DSS) was required. Accordingly, to analyze the temporal relationship between neutrophil functions and progression to severe disease, we selected a subsample of 18 DHF/DSS cases with serum samples collected on day 3 post-fever onset or earlier. These cases were matched by sex and age with 36 DF cases whose serum samples were also collected during acute infection (day 3 post-fever onset or earlier). This dataset was used to compare neutrophil degranulation among clinical groups and to evaluate the correlation of neutrophil degranulation with the maximum documented thickness of the gallbladder wall. The plot in Fig. 4C includes 7 DF and 10 DHF/DSS with gallbladder thickness information from the PDCS and 66 participants with DF and 43 participants with DHF/DSS from the PDHS.
Data exclusions	Pre-established inclusion and exclusion criteria for participants, including exclusions due to missing data, are detailed in the Methods section. For statistical analysis, outliers were identified and removed using the Interquartile Range (IQR) method. Outliers were defined as values outside the following boundaries: Lower Bound: Q1 – (1.5 × IQR) and Upper Bound: Q3 + (1.5 × IQR). Observations beyond these pre-determined thresholds were excluded from further analysis.
Replication	The predictive potential of IgA antibodies for the development of DHF/DSS was evaluated using two independent antibody binding approaches. First, binding was assessed through the median effective dose (ED50), and second, through antibody avidity analysis. Additionally, two independent statistical methods were employed to confirm the association between IgA antibody levels and the risk of severe dengue disease following a subsequent DENV2 infection. Logistic regression models were used to assess associations between pre-infection antibody levels and severe dengue outcomes. Regularization techniques were applied to refine predictive models and confirm the predictive potential of the identified biomarkers. Neutrophil activation induced by pre-infection serum was evaluated using two independent ex vivo models. Antibody-dependent neutrophil phagocytosis (ADNP) and NETosis assays were performed using neutrophils from independent healthy human donors to assess reproducibility in neutrophil activation experiments. In the first model, beads coupled to the antigen of interest (E vs NS1) were opsonized with pre-infection serum, and neutrophil phagocytosis was measured using cells from healthy donors (n=2). The second model involved wells coated with the antigen of interest (E vs NS1) and opsonized with either IgA-depleted or mock-depleted serum, with NET formation quantified via a fluorescent assay. This experiment was performed twice, but one replicate was excluded due to high background signal in the condition with medium alone. In both experiments, neutrophils from healthy donors were stimulated with pre-infection serum from 14 individuals who developed DF and 10 who progressed to DHF/DSS. Markers of neutrophil degranulation during acute infection were measured via ELISA using two technical replicates per sample. In addition, the findings were corroborated using two independent studies: the Pediatric Dengue Cohort Study (PDCS) and the Pediatric Dengue Hospital-based Study (PDHS) in Nicaragua. We employed different laboratory assays to measure neutrophil activation markers (e.g., myeloperoxidase [MPO], neutrophil elastase [NE], lactoferrin, and matrix metalloproteinase-9 [MMP-9]). Both studies were assessed independently to validate early neutrophil activation markers as predictors of vascular leakage and disease progression and their correlation with antibody binding during acute infection.

Randomization	N/A
Blinding	Fluorescence-based neutrophil assays were conducted with image acquisition performed by an operator blinded to the clinical outcomes of the samples.

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
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	Antibody subclass/isotype binding was detected using PE-coupled mouse anti-human detection antibodies against IgA (SouthernBiotech #2050-09), IgM (SouthernBiotech #9020-09), total IgG (SouthernBiotech #9040-09), IgG1 (Southern-Biotech #9052-09), IgG2 (SouthernBiotech #9060-09), IgG3 (Southern-Biotech #9210-09), and IgG4 (SouthernBiotech #9200-09)
Validation	All the antibodies used were validated for the species and application by the manufacturer and in previous studies (DOI: 10.1126/scitranslmed.abm3151; doi: 10.1016/S1473-3099(24)00566-8)

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A