Measuring anti-dengue IgG before vaccination

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Measuring anti-dengue IgG before vaccination

Healthy 46 years old man

attending the travel medicine department

to discuss a possible dengue vaccination.

Measuring anti-dengue IgG before vaccination

The Swiss Expert Committee for Travel Medicine (ECTM) assessed available data for the Qdenga® vaccine and issues the following recommendations:

1. Vaccination against dengue fever virus with Qdenga® in persons with no previous dengue fever infection is not recommended.

2. Vaccination with Qdenga® can be recommended for travellers aged 6 years and older who have evidence of previous dengue infection, defined as i) a laboratory confirmed dengue infection (PCR, antigen or seroconversion) or ii) a compatible history of dengue infection with a positive IgG serological test. AND will be exposed in a region with significant dengue transmission.

Measuring anti-dengue IgG before vaccination

Laboratory confirmed dengue infection? No

Possible exposure to Dengue Virus? Yes
many years living in Singapore, Madagascar, Honduras, Tanzania and annual trips to Costa Rica lasting one month

Dengue Serology with Lateral flow test:
NS1-Ag, IgG and IgM negative

Dengue Serology with the ELISA of Euroimmun:
IgG Index 1.3 = weakly positive (0.8-<1.1 = indeterminate; ≥ 1.1 positive)
IgM Index <0.8 = negative

Measuring anti-dengue IgG before vaccination

What does this result mean?

Did our patient have a dengue virus infection in the past?

Measuring anti-dengue IgG before vaccination
The Problem of Flaviviruses IgG cross-reactions

Table 2
Detection of IgM and IgG antibody against flaviviruses from the sera of patients with dengue.

Sample ID	Days after onset	Indices in IgM-ELISA					Indices in IgG-ELISA	
		DENV[a]	ZIKV[a]	JEV[b]	WNV[a]	TBEV[a]	DENV[a]	TBEV[a]
1/1s	1	0.36	0.25	0.60	0.14	0.16	0.11	0.09
1/2s	25	3.44	0.22	1.64	0.43	0.62	2.12	6.70
1/3s	106	0.78	0.23	0.88	0.25	0.26	2.36	5.72
2/1s	3	0.42	0.17	0.89	0.20	0.14	0.19	0.09
2/2s	8	2.32	0.16	1.30	0.45	0.18	2.18	6.61
2/3s	18	3.21	0.20	2.02	0.66	0.26	2.45	7.17
3/1s	5	2.75	0.20	1.04	0.32	0.19	1.83	5.12
3/2s	12	4.49	0.21	2.56	0.76	0.43	2.89	7.26
4/1s	0	0.42	0.19	0.82	0.14	0.11	1.17	0.53
4/2s	7	5.27	0.17	7.34	3.12	0.23	2.30	7.29
5/1s	3	0.53	1.73	0.99	0.13	0.23	0.49	0.23
5/2s	18	5.73	1.47	6.40	2.52	1.26	2.43	7.59
6/1s	3	0.31	0.21	0.77	0.14	0.16	0.20	0.11
6/2s	16	5.89	0.21	1.56	0.39	0.19	2.55	7.36
7/1s	1	0.34	0.10	0.60	0.15	0.14	0.14	0.09
7/2s	7	5.65	0.25	1.88	0.71	0.91	0.52	0.09
Positive[c]		9	2	7	2	1	10	9
Equivocal[d]		0	0	0	0	1	0	0

[a] Interpretations of the ELISA index: <0.90 negative; 0.90–1.10 equivocal; 1.10 < positive.
[b] The samples are interpreted to be positive if the index is above 1.50.
[c] The number of positive samples.
[d] The number of equivocal samples.

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The Problem of Flaviviruses IgG cross-reactions

Kreuzreaktivität: Die Qualität des verwendeten Antigens garantiert eine hohe Spezifität des ELISA. Aufgrund der hohen Homologien des Zielantigens innerhalb der Gattung der Flaviviren können Kreuzreaktionen dennoch nicht vollständig ausgeschlossen werden. Zur Überprüfung der Kreuzreaktivität wurden Seren von Patienten nach Impfung mit FSME- oder Gelbfieberimpfstoffen sowie von Patienten mit HCV-, West-Nil-Viren- oder Zika-Viren-Infektionen untersucht. Ausgeprägte Kreuzreaktivitäten bestehen vor allem mit Anti-Zika-Viren-IgG-Antikörpern. Es ist zu berücksichtigen, dass Doppelinfektionen besonders in Endemiegebieten möglich sind oder eine Infektion mit einem anderen Flavivirus zu einem früheren Zeitpunkt stattgefunden haben kann. Positive Befunde resultieren dann nicht aus einer Kreuzreaktivität der jeweiligen Antikörper.

Antikörper gegen	n	Anti-Dengue-Viren-Typ-1-4-ELISA (IgG) positiv
FSME-Viren	25	32,0 %
Gelbfieber-Viren	12	0,0 %
Hepatitis-C-Viren	6	0,0 %
West-Nil-Viren	40	50,0 %
Zika-Viren	12	100,0 %

Euroimmun package insert

Measuring anti-dengue IgG before vaccination
Specificity and sensitivity of the tests used

The Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP) recommended that dengue pre-vaccination screening tests for Dengvaxia administration have at least 98% specificity and 75% sensitivity.

The Euroimmun ELISA

TABLE 3 Specificity and sensitivity of commercial anti-DENV IgG tests used to determine previous dengue infection in 400 healthy individuals with specimens classified by virus neutralization and CDC DENV IgG ELISA

Test	% Specificity [95% CI]		% Cross-reactivity [95% CI]	% Sensitivity [95% CI]			
	UNEXPOSED + ZIKV PRIMARY $N = 207$	UNEXPOSED $N = 151$	ZIKV PRIMARY $N = 56$	ALL DENV $N = 193$	MONOTYPIC DENV $N = 36$	MULTITYPIC DENV $N = 77$	MULTI-FLAVIVIRUS $N = 80$
Euroimmun anti-DENV NS1 Type 1–4 ELISA IgG ELISA	97.1 [93.8–98.9]	100 [97.6–100.0]	10.7 [4.0–21.9]	84.5 [78.6–89.3]	55.6 [38.1–72.1]	92.2 [83.8–97.1]	90.0 [81.2–95.6]
Euroimmun anti-DENV Type 1–4 ELISA IgG ELISA	91.8 [87.2–95.1]	100 [97.6–100.0]	30.4 [18.8–44.1]	91.2 [86.3–94.8]	72.2 [54.8–85.8]	96.1 [89.0–99.2]	95.0 [87.7–98.6]

91.8%

33.4%

Medina FA et al., J of Clin Microbiol, 2024

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Returning to our patient…

What about other flaviviruses?

YFV Vaccine: 2003 and 2016

JEV Vaccine: Sept. and Oct. 2016, Booster 2022

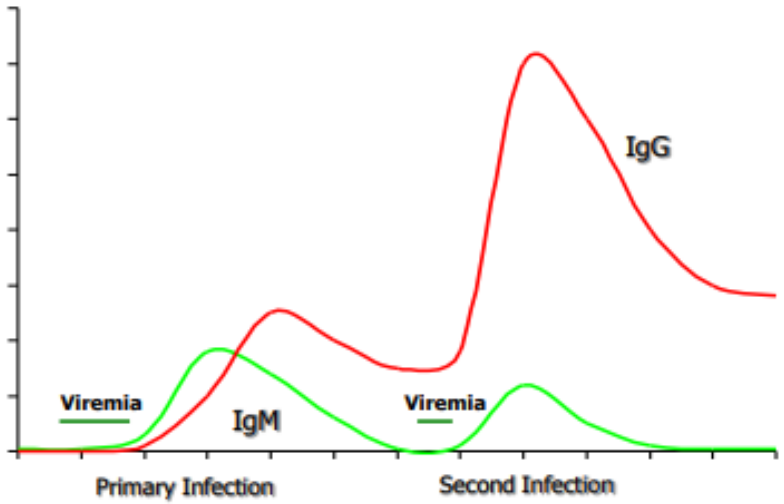
TBEV Vaccine: 2017

Previous asymptomatic Zika infection?

Measuring anti-dengue IgG before vaccination

- Positive IgG because of a previous Dengue infection
- Positive IgG because of a cross-reaction (infection or vaccine) with other flaviviruses
- Negative (or very low) IgG maybe don't exclude a previous Dengue infections if the infection ist very old (Steinke J. et al. European Journal of Clinical Microbiology & Infectious Diseases, 2024)

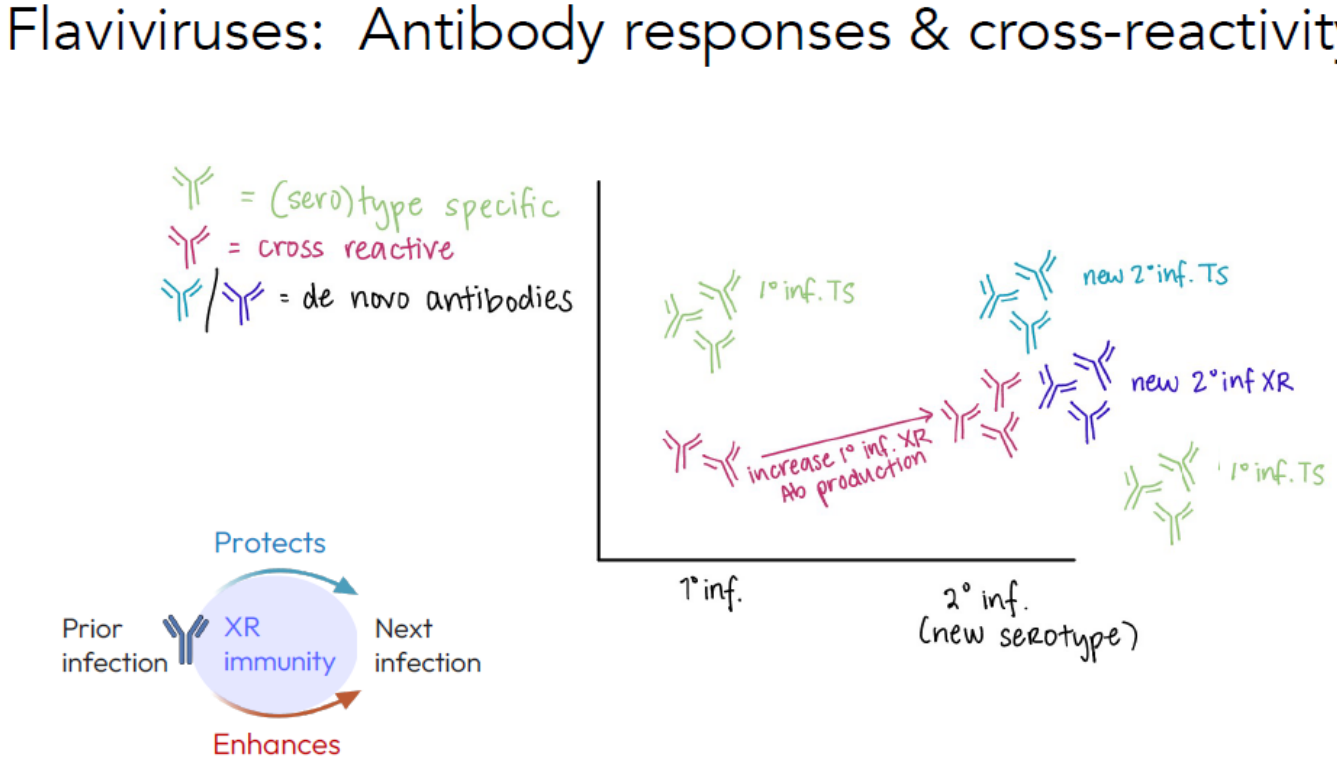
Measuring anti-dengue IgG before vaccination



IgG = immunoglobulin G; IgM = immunoglobulin M

WHO (2012) Handbook for clinical management of dengue

Measuring anti-dengue IgG before vaccination



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What is the meaning of the weakly positive IgG in our patient?

Dengue Virus ELISA IgG (EIA)	1.3	Index	<0.8	Tecan Sunris
Qualifikation, IgG	positiv			Man. Methode

Kommentar

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Wert knapp über dem Cutoff.
Isoliert positive Dengue IgG. Es kann sich um eine früher
durchgemachte Infektion, eine Reinfektion oder eine
Kreuzreaktion mit anderen Flaviviren (z.B. FSME, Gelbfieber,
West Nile, Zika) handeln.
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Value just above the cut-off. This may be due to a previous infection, a reinfection or a cross-reaction with other flaviviruses.

Measuring anti-dengue IgG before vaccination

What is the meaning of weakly positive IgG?

Why are the IgG only weakly positive despite the exposure to many flaviviruses (at least vaccine)?

Would you vaccinate our patient with Qdenga®?

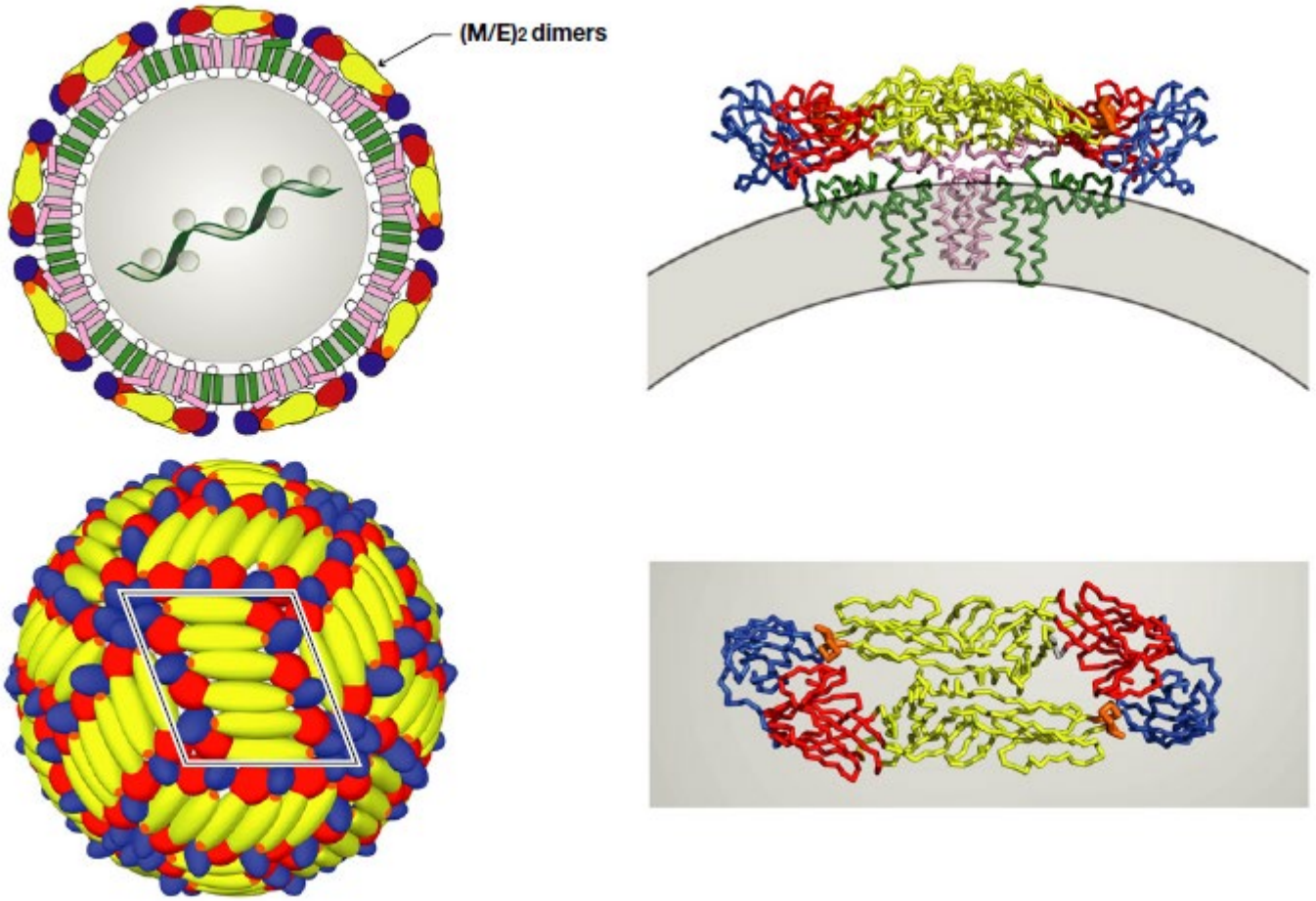
Thank you for your attention

Questions?

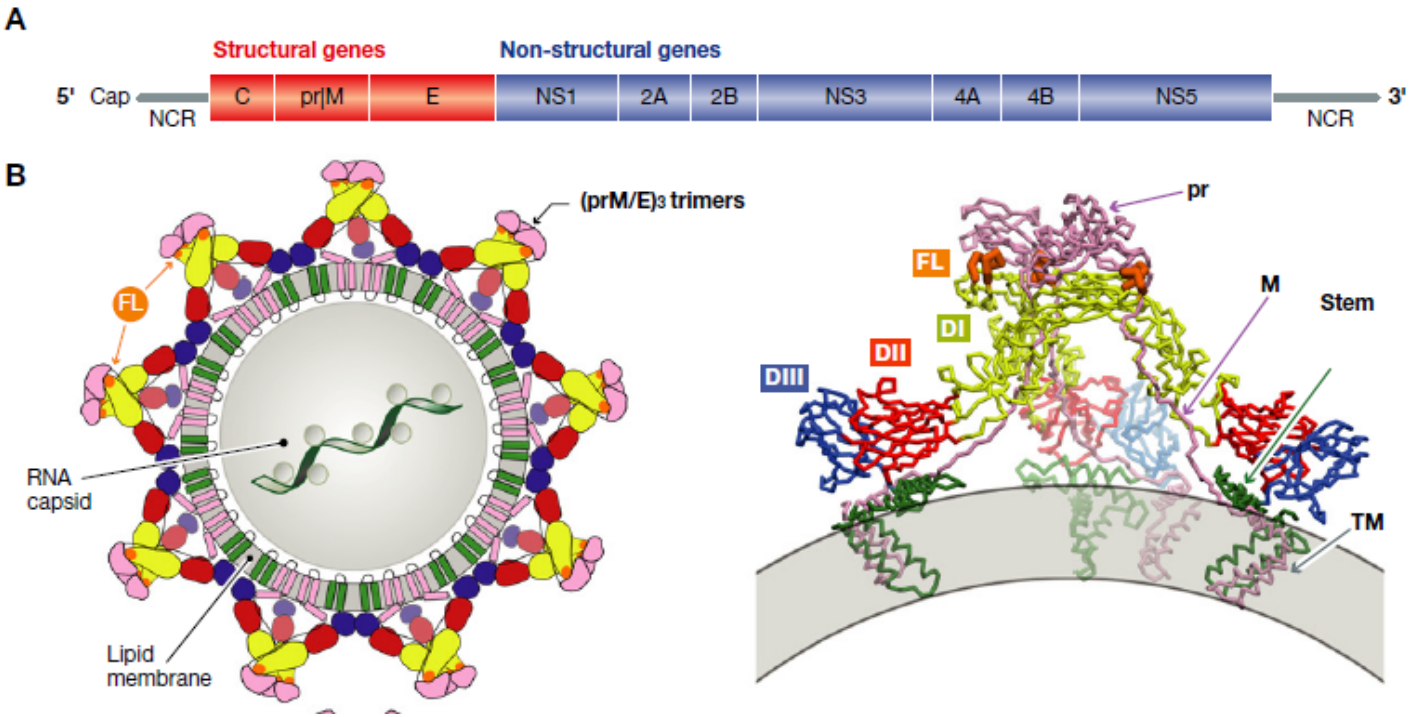
Measuring anti-dengue IgG before vaccination

In secondary infections with dengue or other flaviviruses, the dominance of Fusion loop epitopes (FLE) antibodies is likely to be a consequence of **the original antigenic sin phenomenon** that leads to preferential boosting of pre-existing cross-reactive antibodies upon sequential infections or immunizations with antigenically related molecules.

Rey FA et al., EMBO reports, 2018



Rey FA et al., EMBO reports, 2018

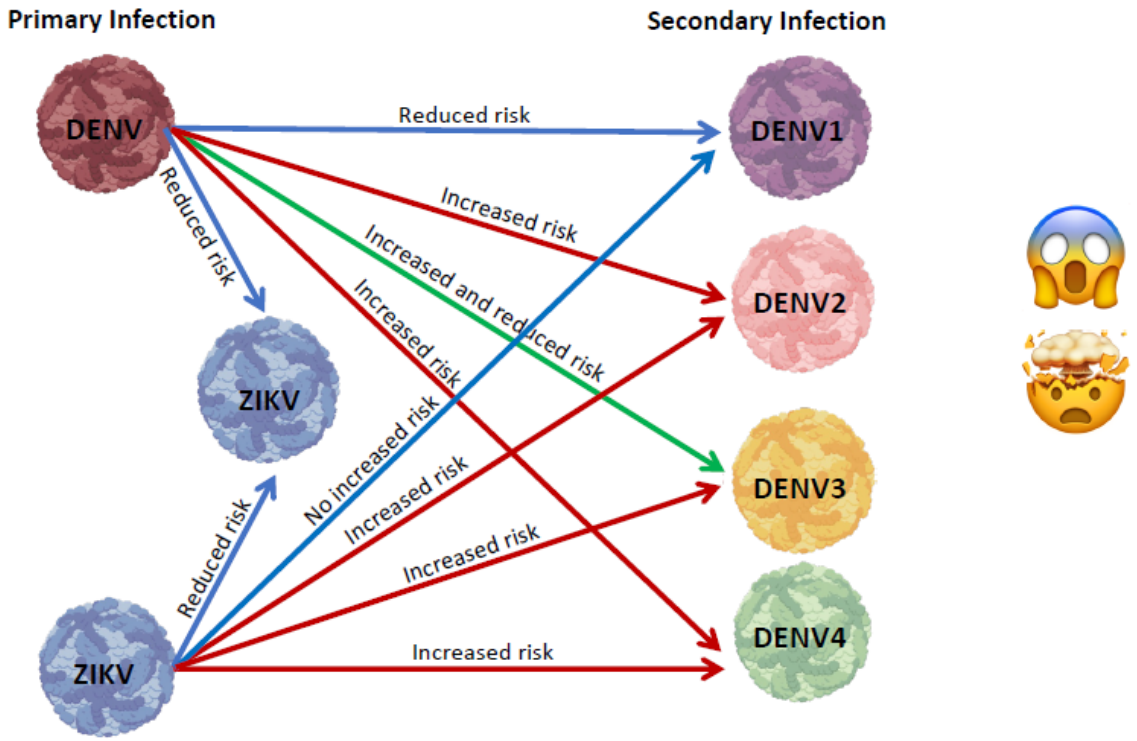


Rey FA et al., EMBO reports, 2018

Following YF17D vaccination, TBEV-pre-vaccinated individuals develop high amounts of cross-reactive IgG antibodies with poor neutralizing capacity. In contrast, TBEV-unvaccinated individuals elicit a non-cross-reacting neutralizing response. Using YF17D envelope protein mutants displaying different epitopes, we identify quaternary dimeric epitopes as the primary target of neutralizing antibodies. Additionally, TBEV-pre-vaccination skews the IgG response towards the pan-flavivirus fusion loop epitope (FLE), capable of mediating ADE of dengue and Zika virus infections in vitro. Together, we propose that YF17D vaccination conceals the FLE in individuals without prior flavivirus exposure but favors a cross-reactive IgG response in TBEV-prevaccinated recipients directed to the FLE with potential to enhance dengue virus infection.

Antonio Santos-Peral et al., Nature Communications | (2024) 15:1696 14
https://doi.org/10.1038/s41467-024-45806-x

Summary: DENV-ZIKV interactions



Katzelnick et al. (2020) *Curr. Opin. Virol.*; Katzelnick et al (2020) *Science*; Zambrana et al. (2024) *Sci Transl Med*

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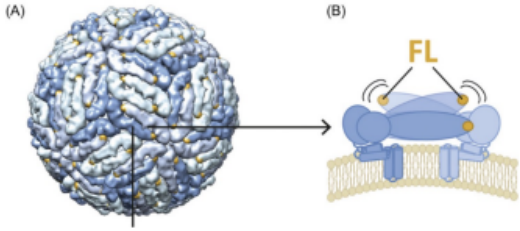
Infection outcome is influenced by the balance between enhancing & protective cross-reactive antibodies

- Magnitude
- Epitope / Stoichiometry
- Function :
  - Neutralization (E)
  - Fc mediated (E + NS1)
  - Prevent internalization (NS1)

anti-Fusion Loop (FL) antibodies



→ Fusion loop = 180 copies /virion mediates virus fusion with cell membrane

→ FL is conserved in all flaviviruses

→ FL neutralizing only if in high quantity

→ Mediates ADE *in vitro* and *in vivo*

Pierson and Diamond *Prog Mol Biol Transl Sci* 2015; de Alwis et al. *PLoS Pathog* 2014; Roßbacher et al, *J Med Virol.* 2023

Conclusions

- Complex epidemiological & immunological interactions of ZIKV DENV1-4
- ZIKV can enhance DENV2 infection due to key immunological determinants
  - pr Abs able to cross-react with DENV2
  - The majority of the DENV2 XR Abs binding to E are FL-Abs and weakly neutralizing
  - Upon DENV2 infection, the majority of the predominant Ab clone recalled from prior ZIKV infection derived-memory B cells fail to neutralize DENV2
- Order of infection modulates the antibody response differently: Sequential DENV2⇌ZIKV infections lead to different subsequent infection outcomes
- DENV serotypes are different viruses with distinct clinical and immunological profiles
- Need to take flavivirus, serotype, and order of infection and vaccination into account in repeat infections due to imprinting

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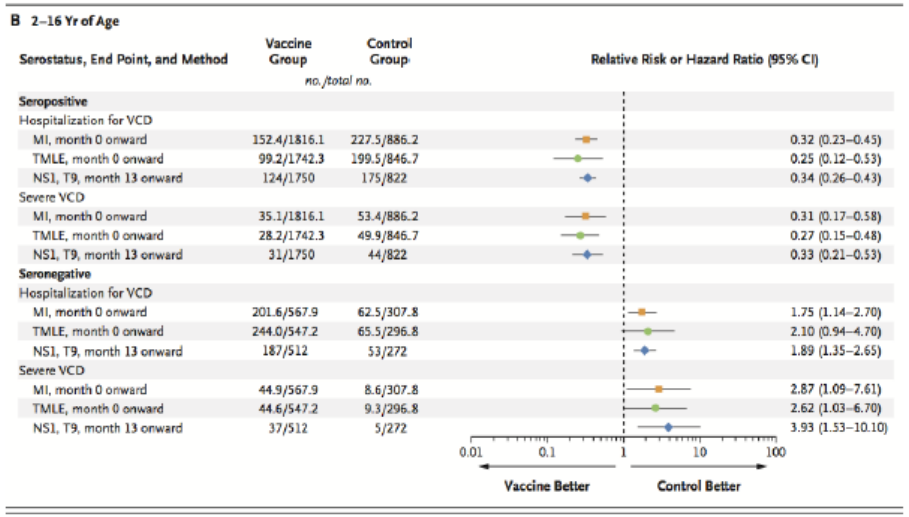
Implications for vaccines

- Re-analysis of the Dengvaxia trial data revealed elevated risk of hospitalized and severe dengue for vaccinated seronegatives

The risk ratio for signs and symptoms associated with vascular leakage syndrome was higher in vaccinated seronegatives

Sridhar et al. (2018) *NEJM*



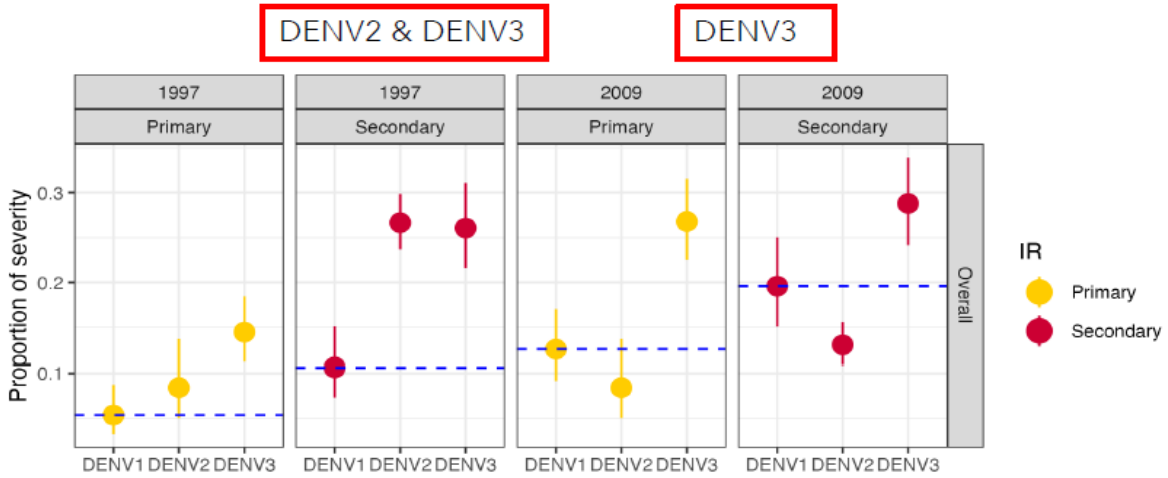
B 2–16 Yr of Age

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)

- Prior flavivirus immunity (TBE vaccine) skews the yellow fever vaccine response to cross-reactive antibodies with potential to enhance DENV infection

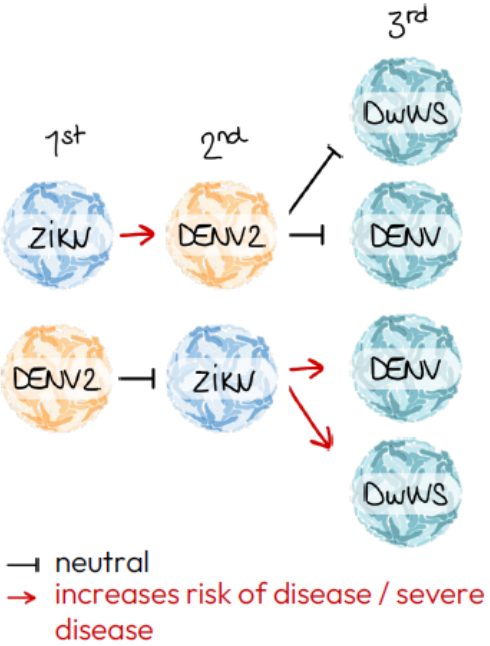
Santos-Peral et al. (2024) *Nat Commun*

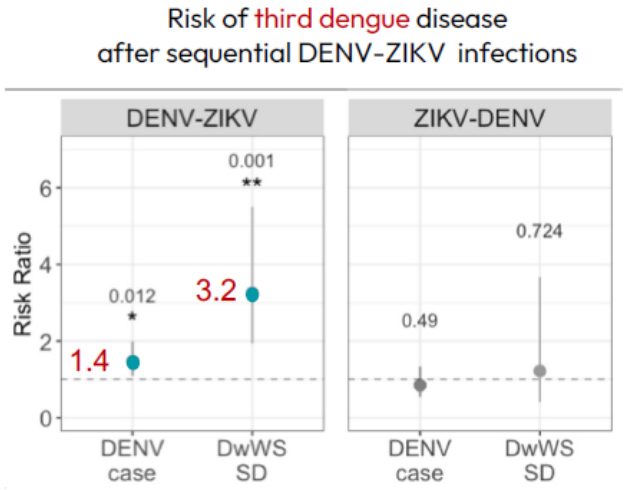
Severe dengue by immune status and serotype, Nicaragua 2004-2022



Narvaez et al. (2025) PLoS Negl Trop Dis

ZIKV & DENV asymmetrical risk of disease are inverted after sequential DENV2⇌ZIKV infections





Zambrana* Hasund* Aogo* et al. (2024) *Sci. Transl. Med.*

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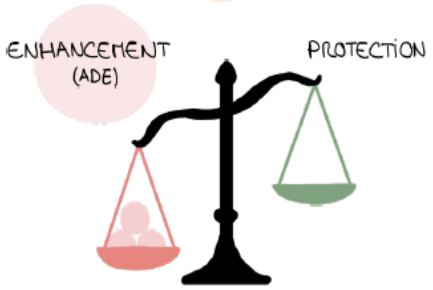
Conclusion



→ pr Abs able to cross-react with DENV2

→ The majority of the DENV2 XR Abs binding to the Envelope are FL-Abs

\+ Weak DENV2 neutralization
(i.e., The magnitude and/or quality of XR Abs are likely insufficient to prevent DENV2 infection)

→ Upon DENV2 infection, the majority of the predominant Ab clone recalled from prior ZIKV infection derived-memory B fail to neutralize DENV2

DENV2 XR Abs : DENV2 cross-reactive antibodies

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ZIKV & DENV asymmetrical risk of disease are inverted after sequential DENV2⇌ZIKV infections

- The neutralization potency against heterotypic DENV serotypes is greater after ZIKV-DENV2 compared to DENV2-ZIKV
- The magnitude of EDIII-specific IgG cross-reactive to heterotypic DENV serotypes is higher after ZIKV-DENV2 infection
- The composition of NS1-specific IgG cross-reactive to heterotypic DENV serotypes is strongly affected by the order of DENV2 ⇌ ZIKV infections

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The Problem of Flaviviruses IgG cross-reactions

TBEV positive Sample tested for Dengue for Evaluation

Antikörper		
Flaviviren: europäische virale Zeckenenzephalitis (FSME)		
IgG EIA	>3000.0	POSITIV
1)2)IgM EIA	30.2	POSITIV
Dieses Resultat spricht für eine kürzlich erworbene Infektion, falls nicht in letzter Zeit geimpft wurde.		
Flaviviren: Gelbfieber (YF), Dengue (DF), West Nile fever (WNF)		
Dengue IgG EIA	4.1	POSITIV
Dengue IgM EIA	<0.8	negativ

Returning from Brazil with Flu-like symptoms, Zika or Dengue?

Antikörper				
Flaviviren: Gelbfieber (YF), Dengue (DF), West Nile fever (WNF)				
Dengue IgG EIA	3.4	POSITIV	0.8	Index
Leicht erhöhte IgGs: Kreuzreaktionen mit anderen Flaviviren oder eine Interferenz bei einer akuten Plasmodien-Infektion (auf Erfahrung beruhend) möglich. Wir empfehlen eine serologische Kontrolle in 10 Tagen zur Erfassung eines IgG Titeranstiegs.				
Dengue IgM EIA	0.8	negativ	0.8	Index
Flaviviren: Zika Virus				
Zika Virus IgG EIA	4.7	POSITIV	0.8	Index
Leicht erhöhte IgGs: Kreuzreaktionen mit anderen Flaviviren oder eine Interferenz bei einer akuten Plasmodien-Infektion (auf Erfahrung beruhend) möglich. Wir empfehlen eine serologische Kontrolle in 10 Tagen zur Erfassung eines IgG Titeranstiegs.				
Zika Virus IgM EIA	<0.8	negativ	0.8	Index