ZIKA RNA PERSISTENCE IN BLOOD AND BODY FLUIDS AND CLINICAL OUTCOMES IN INFECTED BLOOD DONORS

RECIPIENT EPIDEMIOLOGY AND DONOR EVALUATION STUDY-III

Mars Stone, Sonia Bakkour, Tzong-Hae Lee, Marion Lanteri, Graham Simmons, Don Brambilla, Jose Orlando Alsina, Phillip Williamson, Rita Reik, Susan Galel, Jeff Linnen, Steve Kleinman and Michael Busch for the NHLBI Recipient Epidemiology and Donor Evaluation Study-III (REDS-III)

AABB Plenary Session
October 9, 2017
Faculty Disclosure

(In compliance with ACCME policy, AABB requires the following disclosures to the session audience)

• Blood Systems Research Institute is the confirmatory laboratory for both Roche and Grifols Zika INDs
• I am an employee of BSRI
• Roche and Grifols Zika RNA assays are investigational and not commercially available
• I intend to discuss off-label uses of the Grifols Zika TMA assay (application to whole blood samples)
Objectives

• Present findings from follow-up studies of ZIKV RNA positive blood donors, including detection of ZIKV RNA in blood compartments and body fluids and Zika antibody persistence

• Discuss implications of Zika RNA persistence in whole blood relevant to blood screening

• Discuss clinical outcomes in longitudinally followed Zika+ donors
US Natural History Cohort of Zika Virus (ZIKV) RNA Positive Blood Donors

- **Study Design:** Natural history cohort of ZIKV NAT-positive blood donors followed prospectively for 12 months (index + 7 follow-up visits)
- **When:** Launched in June 2016 with accrual through Sept 2018
  - Extended 1 year in anticipation of 2018 Zika outbreak
- **Where:** Puerto Rico, OneBlood, BSI, NYBC, ARC
- **Sample size:** 130 ZIKV+ donors
  - (80 DENV Ab+; 50 DENV Ab-)
US Natural History Cohort of Zika Virus (ZIKV) RNA Positive Blood Donors

Objectives:

▪ Evolution of viral and immunological markers over time
▪ Distribution and compartmentalization in blood and body fluids
▪ Evaluate the viral and immune mechanisms leading to viral clearance or clinical pathogenesis
▪ Evaluate clinical outcomes post donation
▪ Establish a sharable biorepository
  ▪ Characterize the performance of existing and future assays and provide standards for assay development
  ▪ Working with government agencies, industry and academic partners
Study visits and cohort (56 enrolled to date)

Visits completed at each time point

<table>
<thead>
<tr>
<th>Visit #</th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
<th>Visit 5</th>
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Gender and Dengue status

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<th>MALES (38)</th>
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<tr>
<td></td>
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<td>DENV-</td>
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Enrollment per site

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<tr>
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<th>BSSM (PR)</th>
<th>OneBlood</th>
<th>BSI</th>
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June 2016 - Jan 2017
12 months follow up with focus on potential for reinfection

- Allow characterization of humoral and cellular immunity
  - Compare natural infection vs vaccine immune response
- Discriminate recent vs remote infections
  - Facilitate development of ZIKV incidence assays to discriminate recent vs remote infections
  - Monitoring of pregnant women and travellers
- Detection of potential ZIKV reinfections
  - 30-40% exposed in endemic areas in 2016 season
  - Resistant to reinfection = epidemic burn out?
  - Reinfection possible = vaccine efficacy without sterilizing immunity
Higher levels of ZIKV RNA in red cells vs plasma in index donation samples after IgM seroconversion
Longer persistence of ZIKV RNA in whole blood and RBC blood compartments than in plasma and body fluids.
Increased sensitivity detection of ZIKV RNA in plasma and whole blood by blood screening NAT assays relative to confirmatory qPCR/VL assay

Grifols testing performed only on follow up

<table>
<thead>
<tr>
<th>Grifols TMA</th>
<th>BSRI confirmatory qPCR</th>
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<tr>
<td>LOD95</td>
<td>7.5 (4.1, 11.3)</td>
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<tr>
<td>LOD50</td>
<td>1.5 (1.1, 2.1)</td>
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<tr>
<td>LOD95</td>
<td>109 [56.4, 176]</td>
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<tr>
<td>LOD50</td>
<td>15.8 [11.2, 22.2]</td>
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</table>
Implications of Zika persistence in blood compartments and body fluids

1. For acute infection, the use of whole blood extends the period of diagnosis
2. Impact on donation policy: to extend deferral period or consider NAT testing whole blood
3. Consideration for solid organ donation with potential reservoir for viral replication
4. Could testing whole blood or RBC be used as proxy for persistence in semen and sexual transmission risk?
Is RBC-associated ZIKV RNA infectious?

1. There has been no documentation of infectious RBC associated virus after plasma RNA clearance

2. Attempts at inoculating ZIKV RNA+ RBC
   - Onto susceptible cell lines
   - Into IFN- knockout mice
   - Feeding onto Aedes mosquitos
   - Infection of macaques and MID in progress

3. Despite huge epidemics in Latin America and Puerto Rico and French Caribbean Islands (with routine screening), no cases of TT linked to RBC transfusions tested plasma NAT- have been detected

Tentatively concluding:

✓ ZIKV RNA that likely became associated with erythroblasts in acute infection is not infectious
✓ plasma NAT screening is likely sufficient
Zika Ab response is brisk but wanes and weaker when DENV Ab-

- Strong, but rapidly waning neutralizing response in apparent secondary response with strong DENV cross-neutralizing responses
Simmons stage of infection at index donation for 53 REDSIII enrolled donors

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<th>Column1</th>
<th>Serology</th>
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<tr>
<td>Stage I</td>
<td>IgM-</td>
<td>VL &lt;300 IU/ml</td>
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<tr>
<td>Stage II</td>
<td>IgM-</td>
<td>VL ≥300 IU/ml</td>
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<td>Stage III</td>
<td>IgM+/eq</td>
<td>VL ≥300 IU/ml</td>
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<tr>
<td>Stage IV</td>
<td>IgM+/eq</td>
<td>VL &lt;300 IU/ml</td>
<td>higher index VL, RR+, FU R+</td>
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<tr>
<td>Stage IV/V</td>
<td>IgM+/eq</td>
<td>VL &lt;300 IU/ml</td>
<td>incomplete index/fu NAT</td>
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<tr>
<td>Stage V</td>
<td>IgM+/eq</td>
<td>VL &lt;300 IU/ml</td>
<td>index RR-</td>
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</table>
### Symptoms in ZIKV+ donors sorted stage of infection at index donation

#### Pre-IgM (Simmons Stage I/II)
- 15/23 (65%) with >3/6 symptoms
- 4/23 (17%) asymptomatic

#### Post IgM/IgG (Simmons Stage III/IV)
- 8/30 (27%) with >3/6 symptoms
- 9/30 (30%) asymptomatic

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**Symptoms:**
- A=Fever
- B=Rash
- C=Joint Pain or Bone Pain
- D=Body Pain or Muscle pain
- E=Painful Eyes or Red eyes
- F=Headache

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<th>All symptoms</th>
<th>Five symptoms</th>
<th>Four symptoms</th>
<th>Three Symptoms</th>
<th>Two symptoms</th>
<th>One symptom</th>
<th>No Symptoms</th>
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Summary

- Longer persistence of ZIKV RNA in whole blood and RBC blood compartments than in plasma and body fluids
- No evidence of infectious RBC associated virus after plasma RNA clearance
- IgM/IgG and neutralizing Ab responses are brisk but decline
  - Implications for durable and sterilizing immunity
- 65% of donors detected in acute infection are symptomatic vs 27% of later stage donors
- Epidemics may:
  - come quickly – be prepared to respond
  - resolve quickly - leaving scientific and blood policy questions unanswered
Acknowledgements

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  • Don Brambilla
  • Marian Sullivan

• NHLBI
  • Simone Glynn

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  • Steve Kleinman

• REDS-III Contract

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  • Tony Hardiman

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  • Jeff Linnen
  • Kui Gao

• CTS
  • Phillip Williamson

• OneBlood
  • Rita Reik

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  • Jose Orlando Alsina

• UC Davis
  • Koen Van Rompay
  • Lark Coffey

• REDS-III ZIKV Oversight Committee
  • Jay Epstein, FDA
  • Hira Nakhasi, FDA
  • Matt Kuehnert, CDC
  • Lyle Petersen, CDC
  • Brad Biggerstaff, CDC

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  - Chris McClure

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  - Brad Biggerstaff, CDC

- RESEARCH-III Chair
  - Steve Kleinman

- Funding: NHLBI REDS-III, CDC, BARDA
Analyses in progress to estimate RNA persistence

- **Estimation of Roche NAT detection**
  - Puerto Rico donors pre-IgM at index with IND follow up
  - Estimate date of Zika virus infection using macaque acute infection viremia data
  - Observed conversion from NAT reactivity to non-reactivity to estimate NAT reactivity duration

- **Estimation of RNA persistence in REDSIII follow up**
  - Serial longitudinal RNA detection data from 53 donors
  - Infer duration of RNA persistence in blood compartments and body fluids normalized to date of infection
A single mutation in the prM protein of Zika virus contributes to fetal microcephaly

by Ling Yuan, Xing-Yao Huang, Zhong-Yu Liu, Feng Zhang, Xing-Liang Zhu, Jiu-Yang Yu, Xue Ji, Yan-Peng Xu, Guanghui Li, Cui Li, Hong-Jiang Wang, Yong-Qiang Deng, Menghua Wu, Meng-Li Cheng, Qing Ye, Dong-Yang Xie, Xiao-Feng Li, Xiangxi Wang, Weifeng Shi, Baoyang Hu, Pei-Yong Shi, Zhiheng Xu, and Cheng-Feng Qin

Science
Volume ():eaam7120
September 28, 2017

Although it may cause microcephaly when injected IC directly into fetal brains, it alone doesn’t explain the low and regionally-variable incidence of microcephaly in humans.
Fig. 1 Neurovirulence phenotypes of the contemporary ZIKV strains and its ancestral Asian strain.

Ling Yuan et al. Science 2017;science.aam7120
One of the probable evolutions could have been in virus concentration levels. Indeed, in April, an international group of scientists including Singaporean researchers Julien Pompon, Menchie Manuel, Jun Hao Tan and October Sessions, at the Duke-NUS Signature Research Program noted a key difference between the South American and the Asian strains. By feeding mosquitoes Zika-infected blood, the consortium found that the Americas strain of Zika is more effectively transmitted than the Polynesian strain by Aedes aegypti— showing in the mosquitoes’ saliva faster and at significantly higher concentrations.

The S139N mutation in prM described in this paper occurred in pre-2013 Asian lineage ZIKV that was a founder of all American outbreak strains, so it is in all the strains, including the Brazil 2015 strain you provided that we are using in experimental systems.

Zika virus (ZIKV) has evolved into a global health threat due to its unexpected causal link to microcephaly. Phylogenetic analysis reveals that contemporary epidemic strains have accumulated multiple substitutions from their Asian ancestor. Here, we show that a single serine to asparagine substitution (S139N) in the viral polyprotein substantially increased ZIKV infectivity in both human and mouse neural progenitor cells (NPCs), led to more significant microcephaly in the mouse fetus, and higher mortality in neonatal mice. Evolutionary analysis indicates that the S139N substitution arose before the 2013 outbreak in French Polynesia and has been stably maintained during subsequent spread to the Americas. This functional adaption makes ZIKV more virulent to human NPCs, thus contributing to the increased incidence of microcephaly in recent ZIKV epidemics.
Sharing samples from ZIKV biorepository

Enabling development and optimization of serology assays and pathogenesis, vaccine and cellular immunological studies

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Sample Use</th>
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<td>WHO</td>
<td>International standards for Zika and CHIK</td>
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<td>Charles Chiu</td>
<td>Transcriptomics and deep sequencing</td>
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<tr>
<td>Lyle Peterson - CDC</td>
<td>Zika Dengue metabolomics</td>
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