Positive IgM for Zika virus in the cerebrospinal fluid of 30 neonates with microcephaly in Brazil

The epidemic of microcephaly in Brazil has been declared a Public Health Emergency of International Concern by WHO.[1] The declaration states that a causal relationship between Zika virus infection during pregnancy and microcephaly is strongly suspected, although not yet scientifically proven.[1] The hesitancy to accept causation in the presence of much epidemiological circumstantial evidence is due to the paucity of laboratory confirmation of Zika virus in affected neonates. Here, we report the serological confirmation of Zika virus infection in the CNS of 30 neonates with microcephaly.

From Oct 21 to Oct 30, 2015, we collected blood and cerebrospinal fluid (CSF) samples from 31 neonates with microcephaly in the state of Pernambuco, Brazil, most of whom were born between Sept 12 and Oct 27, 2015, in public maternity hospitals. The samples were collected on average 9 days after birth (range 1–40 days; 20 of 31 neonates in the first week after birth). The neonates were kept in hospital while results from brain imaging and laboratory tests were awaited. Serum and CSF samples were tested by RT-PCR or real-time RT-PCR, or both, for Zika, dengue, and chikungunya genomes. Viral RNA was not detected, probably because infections in the mother occurred at least 6 months before giving birth. The 31 samples of CSF and serum were tested for IgM specific for Zika virus using capture ELISA based on the US Centers for Disease Control and Prevention (CDC) Emergency Use Authorization protocol with reagents from Robert Lanciotti (CDC Fort Collins, CO, USA). Simultaneous tests were done for dengue virus to investigate cross-reaction between these two flaviviruses. The ratio of patient optical density to negative control value (P/N) was calculated (table).[2]

Zika-specific IgM was detected in 30 (97%) of 31 CSF samples and in 28 (90%) of 31 serum samples. One CSF sample tested negative. Monotypic response to Zika virus in the CSF was confirmed by plaque reduction neutralisation test. Since IgM does not cross either the placenta barrier or the blood–brain barrier, the presence of IgM in the CSF indicates that the neonate had the infection in the CNS.[3,4] The finding of Zika-specific IgM in the CSF of those 30 of 31 neonates with brain abnormalities indicates that they had a congenital infection with Zika virus. We believe that this is very strong evidence that the microcephaly was a consequence of Zika virus infection.

We declare no competing interests. We thank Laura C Rodrigues and the Microcephaly Epidemic Research Group for their support.

Published Online
April 18, 2016
http://dx.doi.org/10.1016/S0140-6736(16)30253-7

	Serum IgM		CSF IgM		
	Zika virus	Dengue virus type 1–4 mixture	Zika virus	Dengue virus type 1–4 mixture	Interpretation
1 day	17·0	2·7	12·1	1·5	Positive for Zika virus
1 day	20·6	2·9	16·1	2·4	Positive for Zika virus
1 day	20·6	7·8	14·8	4·2	Zika virus cross-reacting with dengue virus
1 day	5·2	0·7	9·3	1·0	Positive for Zika virus
1 day	8·2	1·7	16·3	3·4	Zika virus cross-reacting with dengue virus
2 days	6·2	0·9	15·0	1·5	Positive for Zika virus
2 days	6·2	0·9	14·5	2·7	Positive for Zika virus
2 days	7·5	0·9	16·1	2·9	Positive for Zika virus
2 days	4·7	0·9	14·2	1·7	Positive for Zika virus
2 days	12·7	1·2	15·9	2·9	Positive for Zika virus
2 days	10·5	1·7	15·8	2·1	Positive for Zika virus
3 days	10·5	1·1	14·8	2·4	Positive for Zika virus
3 days	15·6	2·6	14·8	2·4	Positive for Zika virus
3 days	16·0	1·6	16·4	1·9	Positive for Zika virus
4 days	3·2	0·6	13·5	1·9	Positive for Zika virus
5 days	3·9	0·8	9·3	0·8	Positive for Zika virus
5 days	11·4	5·5	15·5	4·6	Zika virus cross-reacting with dengue virus
7 days	5·9	0·7	13·1	1·2	Positive for Zika virus
7 days	2·1	0·9	15·0	0·9	Positive for Zika virus
7 days	15·4	2·2	13·5	1·6	Positive for Zika virus
8 days	9·6	1·8	15·7	1·7	Positive for Zika virus
10 days	4·0	1·5	14·5	6·6	Zika virus cross-reacting with dengue virus
11 days	0·9	1·8	0·6	1·9	Negative for Zika virus
12 days	16·1	6·2	15·7	5·0	Zika virus cross-reacting with dengue virus
13 days	15·3	2·6	12·1	1·3	Positive for Zika virus
17 days	6·4	1·8	16·1	1·4	Positive for Zika virus
17 days	16·0	2·8	14·8	3·0	Positive for Zika virus
22 days	4·1	1·2	15·5	2·7	Positive for Zika virus
23 days	3·4	2·6	16·1	5·7	Zika virus cross-reacting with dengue virus
36 days	2·1	0·9	15·6	1·9	Positive for Zika virus
40 days	12·2	1·1	13·3	0·8	Positive for Zika virus

ELISA values are patient optical densities divided by negative control densities (P/N); values less than 2 were considered negative, 2–3 equivocal, and more than 3 positive. CSF=cerebrospinal fluid.

Table: **IgM against Zika virus and dengue virus in the serum and CSF of neonates with microcephaly, Pernambuco State, Brazil, 2015, by age (days) at testing**

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1 WHO. WHO Director-General summarizes the outcome of the Emergency Committee regarding clusters of microcephaly and Guillain-Barré syndrome. Feb 1, 2016. http://www.who.int/mediacentre/news/statements/2016/emergency-committee-zika-microcephaly/en/ (accessed Feb 12, 2016).

2 Lanciotti RS, Kosoy OL, Janeen J, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* 2008; **14:** 1232.

3 Carrolla ID, Toovey SA. Dengue fever and pregnancy—a review and comment. *Travel Med Infect Dis* 2007; **5:** 3183.

4 Tunkel AR, Glaser CA, Bloch KC, et al. The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2008; **47:** 303–27.