

# Zika virus and microcephaly: is the correlation causal or coincidental?

Giuseppina Liuzzi, Vincenzo Puro, Simone Lanini, Francesco Vairo, Emanuele Nicastrì, Maria Rosaria Capobianchi, Antonino Di Caro, Mauro Piacentini, Alimuddin Zumla<sup>1</sup>, Giuseppe Ippolito

National Institute for Infectious Diseases "Lazzaro Spallanzani" - IRCCS, Rome, Italy;

<sup>1</sup>Division of Infection and Immunity, University College London, and NIHR Biomedical Research Centre, UCL Hospitals NHS Foundation Trust, London, UK

Public health authorities in Brazil are currently investigating whether the apparent surge in the number of babies born with microcephaly are causally linked with Zika virus (ZIKV) infection during pregnancy. Indeed, by 2015 an increase in the number of infants born with microcephaly was reported in Brazil, a country experiencing since April 2015 the largest known outbreak of ZIKV infection (Petersen *et al.*, 2016). The number of suspected cases of microcephaly has continued to rise in Brazil reaching about 5000 cases since the authorities began investigating the surge.

The World Health Organization Emergency Committee on ZIKV stated that a causal link between ZIKV and microcephaly is "strongly suspected, though not yet scientifically proven" (WHO, 2016a) and declared the cluster of microcephaly cases and other neurological disorders reported in Brazil as a Public Health Emergency of International Concern (WHO, 2016b).

After the suspicion was raised that the increase in birth defects could be attributed to the massive use of the larvicide pyriproxifen, Brazil's health ministry quickly posted a clarification on the use of pyriproxifen, noting that there is no scientific basis for a link between the use of the larvicide and microcephaly and that some cities that have not used it have also reported microcephaly cases.

This temporal increase in cases of microcephaly could also be distorted given both raised awareness and changing definitions of microcephaly over time (Victoria *et al.*, 2016). In fact, out of the 4783 suspect cases of microcephaly reported, only 1103 had complete clinical, laboratory and imaging examinations and 404 of them were classified as confirmed cases of microcephaly: brain abnormalities were detected by imaging in 387 babies and ZIKV was detected in 17 babies, including two fetal losses (Victoria *et al.*, 2016).

Although the findings can at least in part be an overestimation due to diagnostic bias, there has been a huge increase in congenital microcephaly from fewer than 150

cases reported in the country in 2014, and it looks temporally related to the introduction and widespread dissemination of ZIKV infection. Epidemiological investigations are ongoing to assess the degree of association between the infection and microcephaly.

## What is microcephaly?

Microcephaly is a neonatal malformation defined as a head size much smaller than those of other babies of the same age and sex. If this combines with poor brain growth, babies with microcephaly may achieve developmental disabilities. The clinical presentation of microcephaly ranges from mild to severe. There are no specific tests to determine if a baby will be born with microcephaly, but ultrasound scans in the third trimester of pregnancy can sometimes identify the problem (CIDRAP 2016, WHO 2016b).

Microcephaly can result from chromosomal abnormalities, exposure to drugs, alcohol, other environmental toxins, premature fusion of the bones of the skull (craniosynostosis), certain metabolic disorders, and congenital infections (Schuler-Faccini *et al.*, 2016).

One of the causes of microcephaly involves abnormal function of centrosomes (Thornton *et al.*, 2009), normally associated with mitosis, but also involved in other cellular processes including migration, polarity and proper trafficking of vesicles.

Amplification of the number of centrosomes has been revealed to be one of the inducers of microcephaly (Marthiens *et al.*, 2013). In fact, in the context of neural brain development, an increase in centrosomes in mice results in delayed mitosis, increased apoptosis, improper neural stem cell orientation, premature neuronal differentiation, and a decreased number of progenitor cells (Marthiens *et al.*, 2013). The overall effect is reduced brain matter formation, ultimately leading to reduced brain size.

## Other viruses and microcephaly

During human gestation, viruses can cause intrauterine infections associated with pregnancy complications and fetal abnormalities. Common sonographic abnormalities may be indicative of fetal viral infections. Some of the pathognomonic sonographic findings enable diagnosis of a specific congenital syndrome, e.g. ventriculomegaly and intracranial and hepatic calcifications in cytomegalovirus

### Key words:

ZIKA Virus, Microcephaly.

### Corresponding author:

Giuseppe Ippolito

E-mail: giuseppe.ippolito@inmi.it

infection (CMV); eye and cardiac anomalies in congenital rubella (Degani 2006).

CMV infection is a significant cause of brain disorders such as microcephaly, mental retardation, hearing loss, and visual disorders. Despite the numerous descriptions of the typical neuropathologic manifestations of intrauterine CMV infection, few studies have focused on the pathophysiologic mechanisms by which CMV infection affects developing human brains. CMV infects all cell types but shows increased tropism for stem cells/radial glial cells.

Two main factors influence the neuropathologic outcome at this stage: the density of CMV-positive cells and the tropism of CMV for stem/progenitor cells. This suggests that the broad spectrum of CMV-induced brain abnormalities is caused not only by tissue destruction but also by the particular vulnerability of stem cells during early brain development (Teissier *et al.*, 2014).

### The Zika virus

ZIKV is a single stranded RNA arbovirus member of the genus *Flavivirus* and is related to other mosquito-borne viruses such as Dengue, Yellow Fever, Japanese encephalitis and West Nile Fever viruses. Transmission of ZIKV between humans occurs primarily through the bite of an infected female mosquito of the *Aedes* species. Apart from the mosquito-originated inoculation, transmission of ZIKV has been reported to occur via sexual intercourse and blood transfusion; vertical perinatal transmission has also been reported (Petersen *et al.*, 2016).

### The Zika virus and microcephaly

Like CMV, ZIKV may have the potential to infect the fetus and cause neurodevelopmental dysfunctions including microcephaly.

On 17 November 2015, the Brazilian ministry of health reported the presence of ZIKV RNA in amniotic fluid samples collected from two pregnant women from the state of Paraíba, whose foetuses showed microcephaly. The two mothers had symptoms compatible with ZIKV disease at gestation weeks 18 and 19.

Ultrasonography done at gestation week 20 revealed calcifications in the foetuses' brains, and repeated scan at gestation week 28 confirmed the diagnosis of microcephaly. Urine and serum samples from the mothers were negative for ZIKV genome detection at gestation week 28, but amniocenteses were positive with a viral load 10,000 times higher than that normally found in blood from adults with acute infection and exanthema (ECDC 2015). In December 2015, the Brazilian ministry of health reported the presence of ZIKV genome in the blood and tissue samples of a baby from the state of Pará with microcephaly.

The newborn presented microcephaly and other congenital anomalies and died within five minutes of being born. (Pan American Health Organization).

An unusual feature of at least 17 cases of central nervous system malformations in fetuses and infants was reported in the French Polynesian Islands during 2014-2015 coinciding with ZIKV outbreaks (ECDC 2015).

Based on the temporal correlation of these cases with the ZIKV epidemic, the health authorities hypothesize that ZIKV infection may be associated with these abnormalities when mothers are infected during the first or second trimester of pregnancy (ECDC 2015).

In December 2015, tissue samples, brain and other autopsy tissues from two newborns (born at 36 and 38 weeks gestation) with microcephaly who died within 20 hours of birth, a placenta from one of the newborns, and products of conception from two miscarriages (fetal losses at 11 and 13 weeks) were referred to the Center for Disease Control by the state of Rio Grande do Norte in Brazil for histopathologic evaluation and laboratory testing for suspected ZIKV infection.

All four mothers had clinical signs of ZIKV infection, including fever and rash, during the first trimester of pregnancy, but did not have clinical signs of active infection at the time of delivery or miscarriage. The mothers were not tested for antibodies to ZIKV.

This report describes evidence of a link between ZIKV infection and microcephaly and fetal demise, through the detection of viral RNA and antigens in brain tissues from infants with microcephaly and placental tissues from early miscarriages. In fact, specimens from all four cases were positive to ZIKV-specific RT-PCR, and sequence analysis revealed the highest identity with viral strains isolated from Brazil during 2015. In addition, histopathologic findings indicate the presence of ZIKV in fetal tissues.

These findings also suggest that brain and early gestational placental tissue might be the preferred tissues for post-mortem viral diagnosis (Martines 2016).

In their pivotal study published in February 2016, Mlakar *et al.* further supported the association between ZIKV infection and microcephaly, demonstrating severe fetal brain injury in a case of vertically acquired ZIKV infection: electron microscopy analysis of fetal brain revealed the presence of virus particles compatible with ZIKV. In addition, the complete genome sequence was recovered from fetal brain tissue (Mlakar *et al.*, 2016).

### Pathogenetic hypothesis for microcephaly related to Zika virus

In 1952, Dick *et al.*, (Dick *et al.*, 1952, Dick 1952) demonstrated ZIKV tropism for the brain in intraperitoneally infected mice. About 20 years later central nervous system disease was observed in infected mice by Bell *et al.* (Bell *et al.*, 1971). The virus could cross the blood brain barrier and infected both neurons and glia, producing a variety of intracytoplasmic inclusions, which they termed, "virus factories".

These factories originated from the endoplasmic reticulum and associated with other organelles including the nucleus and the mitochondria. Those microscopic observations are also compatible with the induction of autophagy. This is not unexpected, as the induction of autophagy has been observed for other flaviviruses, as a result of the interaction between the virus and the endoplasmic reticulum (Blazquez *et al.*, 2014).

More recently, a potential role for autophagy in ZIKV infection has been shown in cultured human skin cells, where the formation of autophagosomes containing viral capsids was observed (Hamel *et al.*, 2015). This event seems to be relevant for viral replication, as the pharmacological modulation of autophagy might decrease the amount of viral RNA produced in the cultures.

Although autophagy has not been described in Zika-infected neural cells, the evidence obtained in experimentally-infected skin fibroblasts (Hamel *et al.*, 2015) opens a new landscape on the mechanisms of generation of brain

abnormalities, possibly mediated by the viral interference with the physiological process of autophagy. In fact, it is well known that the blockade of autophagy during embryogenesis leads to nervous system defects (Fimia *et al.*, 2007). This provides some evidence to support the involvement of ZIKV in the damage of cells from multiple lineages, including neural cells (Bell *et al.*, 1971).

Besides perturbation of the autophagy pathway, a summary of pathogenetic mechanisms involved in the generation of microcephaly is reported in the section "What is microcephaly?" Although the mechanisms of ZIKV pathogenesis appear to fall in line with the requirements for centrosome abnormalities, there is as yet no evidence to prove culpability.

Future studies need to be performed to definitively establish this link. In particular, vertical transmission of ZIKV as well as any direct or indirect effects of infection on neural development need to be concretely demonstrated. Furthermore, studies should explore other aberrations in fetal development apart from microcephaly.

### Final comments

The ZIKV outbreak in the Americas and the South Pacific region is rapidly evolving, and a major concern is that its spread is likely to continue, as the vector species *Aedes aegypti* and *Aedes albopictus* are widely distributed there. Another concern is the possible spread of the epidemic out of these areas through autochthonous transmission, subsequent to the introduction of cases in European areas where the competent vector is present. *Aedes albopictus* is widely distributed in the Mediterranean basin including Italy.

Although it has not yet been confirmed that the population of mosquitoes living in Europe can effectively transmit the infection, an effective autochthonous transmission of an arbovirus (Chikungunya virus) sustained by *Aedes* mosquitoes already occurred in Italy in 2007 (Bordi *et al.*, 2008).

The return of travelers from ZIKV endemic areas may be a source for autochthonous transmission, especially in the months of increased mosquito activity. Enhanced surveillance and reporting of cases in these regions is recommended (ECDC 2016).

While a significant increase in the number of newborns presenting with a low head circumference seems established in the north-eastern states of Brazil, the magnitude of the increase cannot be precisely estimated. Although the microcephaly cases in Brazil seem to be spatio-temporally associated with the ZIKV outbreak, more robust investigations and research are needed to better understand this potential link. Furthermore dedicated studies, including case-control studies, are also needed to determine the magnitude of the potential risk and identify other possible risk factors.

In the meanwhile, appropriate information should be given to pregnant women traveling in endemic areas about their risk and about the correct diagnostic procedures and surveillance protocols. For the same reason, it is important to advise pregnant women, or women who are planning a pregnancy, to precautionally consider postponing travel to endemic areas. Moreover, some reports strongly support the possibility of transmission through sexual intercourse and blood transfusion (Petersen *et al.*, 2016): therefore, it is also important to inform patients, even with low-grade symptoms, to use condoms in sex-

ual intercourse and to avoid blood donations. Similarly, travellers to endemic areas should be informed about these routes of transmission, in order to reduce at-risk behaviours.

### References

- Bell T.M., Field E.J., Narang H.K. (1971). Zika virus infection of the central nervous system of mice. *Arch Gesamte Virusforsch.* **35**, 183-193.
- Blazquez A.B., Escribano-Romero E., Merino-Ramos T., Saiz J.C., Martín-Acebes M.A. (2014). Stress responses in flavivirus-infected cells: activation of unfolded protein response and autophagy. *Front Microbiol.* **5**, 1-7.
- Bordi L., Carletti F., Castilletti C., Chiappini R., Sambri V., Cavrini F., et al. (2008). Presence of the A226V mutation in autochthonous and imported Italian chikungunya virus strains. *Clin Infect Dis.* **47**, 428-429.
- CIDRAP. (2016). WHO gives overview of Zika impact; Brazil cites 300 more microcephaly cases. (2016). <http://www.cidrap.umn.edu/news-perspective/2016/02/who-gives-overview-zika-impact-brazil-cites-300-more-microcephaly-cases>.
- Degani S. (2006). Sonographic findings in fetal viral infections: a systematic review. *Obstet Gynecol Surv.* **61**, 329-336.
- Dick G.W., Kitchen S.F., Haddock A.J. (1952). Zika virus. I. Isolations and serological specificity. *Trans R Soc Trop Med Hyg.* **46**, 509-520.
- Dick G.W. (1952). Zika virus II. Pathogenicity and physical properties. *Trans R Soc Trop Med Hyg.* **46**, 521-534.
- European Centre for Disease Prevention and Control. (2016). Zika virus epidemic in the Americas: potential association with microcephaly and Guillain-Barré syndrome (first update) 21 January 2016. (accessed 18 Feb, 2016).
- European Centre for Disease Prevention and Control. (2015). Microcephaly in Brazil potentially linked to the Zika virus epidemic [http://ecdc.europa.eu/en/publications/\\_layouts/forms/Publication\\_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1407](http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1407) (accessed 18 Feb, 2016).
- Fimia G.M., Stoykova A., Romagnoli A., Giunta L., Di Bartolomeo S., Nardacci R., et al. (2007). Ambra1 regulates autophagy and development of the nervous system. *Nature.* **447**, 1121-1125.
- Hamel R., Dejarnac O., Wichit S., Ekchariyawat P., Neyret A., Luplertlop N., et al. (2015). Biology of zika virus infection in human skin cells. *J Virol.* **89**, 8880-8896.
- Marthiens V., Rujano M.A., Pennetier C., Tessier S., Paul-Gilloteaux P., Basto R. (2013). Centrosome amplification causes microcephaly. *Nat Cell Biol.* **15**, 731-740.
- Martines R.B., Bhatnagar J., Keating M.K., Silva-Flannery L., Muehlenbachs A., et al. (2016). *Notes from the Field: Evidence of Zika Virus Infection in Brain and Placental Tissues from Two Congenitally Infected Newborns and Two Fetal Losses - Brazil, 2015 MMWR Morb Mortal Wkly Rep Early Release.* **65**, 1-2.
- Mlakar J., Korva M., Tul N., Popović M., Poljšak-Prijatelj M., Mraz J., et al. (2016). Zika Virus Associated with Microcephaly. *N Engl J Med.* Feb 10.
- Pan American Health Organization, World Health Organization. Regional Office for the Americas. (2015) Epidemiological Alert: Neurological syndrome, congenital malformations, and Zika virus infection. Implications for public health in the Americas. Washington: World Health Organization. [http://www.paho.org/hq/index.php?option=com\\_docman&task=doc\\_download&Itemid=&gid=32405&lang=en](http://www.paho.org/hq/index.php?option=com_docman&task=doc_download&Itemid=&gid=32405&lang=en) (accessed 18 Feb, 2016).
- Petersen E., Wilson M.E., Touch S., McCloskey B., Mwaba P., Bates M., et al. (2016). Unexpected and Rapid Spread of Zika Virus in The Americas - Implications for Public Health Preparedness for Mass Gatherings at the 2016 Brazil Olympic Games. *Int J Infect Dis.* **44**, 11-15.
- Schuler-Faccini L., Ribeiro E.M., Feitosa I.M., Horovitz D.D., Cavalcanti D.P., Pessoa A., et al. (2016). Possible Association Between Zika Virus Infection and Microcephaly - Brazil, 2015 MMWR Morb Mortal Wkly Rep. **65**, 59-62.
- Teissier N., Fallet-Bianco C., Delezoide A.L., Laquerrière A., Marcourelles P., Khung-Savatovsky S., et al. (2014). Cytomegalovirus-induced brain malformations in fetuses. *J Neuropathol Exp Neurol.* **73**, 143-158.
- Thornton GK, Woods CG. (2009). Primary microcephaly: do all roads lead to Rome? *Trends Genet.* **25**, 501-510.
- Victora C.G., Schuler-Faccini L., Matijasevich A., Ribeiro E., Pessoa A., Barros F.C. (2016). Microcephaly in Brazil: how to interpret reported numbers? *Lancet.* **387**, 621-624.
- WHO. (2016a). WHO Director-General summarizes the outcome of the Emergency Committee regarding clusters of microcephaly and Guillain-Barré syndrome. 1 February 2016 (<http://www.who.int/mediacentre/news/statements/2016/emergency-committee-zika-microcephaly/en/>) (accessed Feb 20, 2016).
- WHO. (2016b). Emergencies: microcephaly. [www.who.int/emergencies/zika-virus/microcephaly/en/](http://www.who.int/emergencies/zika-virus/microcephaly/en/) (accessed Feb 18, 2016).