Infectious causes of microcephaly: Epidemiology, Pathogenesis, Diagnosis, and Management

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Summary

Microcephaly is an important sign of neurological malformation and predictor of future disability. The recent outbreak of Zika virus and congenital Zika infection has brought the world's attention to the links between infection and microcephaly. However, Zika virus is only one of the infectious causes of microcephaly and, though the contexts in which they occur vary greatly, all are of concern. In this review, we summarise important aspects of the major congenital infections that can cause microcephaly, describing the epidemiology, transmission, clinical features, pathogenesis and long-term consequences. We include the infections that cause substantial impairment: cytomegalovirus, herpes simplex virus, rubella virus, *Toxoplasma gondii*, and Zika virus. We highlight potential issues with the classification of microcephaly and show how some infants affected by congenital infection may be missed or incorrectly diagnosed. While the world's current focus on Zika virus is remarkable, preventing all infectious causes of

microcephaly and appropriately managing its consequences remain important global public health priorities.

Introduction

This review was conducted in response to the recent outbreak of microcephaly associated with congenital Zika virus (ZIKV) infection, first identified in Latin America. Attention has been drawn to ZIKV because the previously unknown congenital syndrome was discovered in a susceptible population and can potentially affect a very large number of infants. ZIKV however is one of a group of infectious diseases that can be transmitted to the fetus and cause microcephaly and the microcephaly is likely to represent the tip of the iceberg in terms of developmental abnormalities, forming part of a syndrome for each infection. Understanding the different causes of microcephaly will help clinicians, researchers, and hopefully policy makers contextualize the recent outbreak and can be applied to help the individual child, as well as to inform appropriate planning of services for the population.

In this article, we review the major congenital infections that can cause microcephaly, focusing on those with the largest disease burden and the strongest evidence for causation: cytomegalovirus (CMV), herpes simplex virus (HSV), rubella virus, *Toxoplasma gondii* (*T. gondii*), and ZIKV. We describe the epidemiology, pathogenesis, transmission, clinical features and long-term disability in childhood. Less frequent causes of primary microcephaly are not considered here. While congenital syphilis has a high disease burden in regions affected by ZIKV and varicella virus has recognised neurotropic properties, published reports describing primary microcephaly in the context of these two infections are scarce.

Microcephaly

Microcephaly is a clinical diagnosis made at or after birth that describes a small head. It does not necessarily mean abnormal brain development and some children with microcephaly are healthy.

An accepted definition of microcephaly is occipito-frontal head circumference (OFC)>2 standard deviations below the median (<-2 *z*-scores), for gestational age and sex in an appropriate healthy reference population, with severe microcephaly defined as <-3 *z*-scores.[1-3] The reference range and population from which a definition is taken can vary, for example country-specific ranges, the World Health Organization (WHO) child-growth ranges for term infants, the Fenton ranges for preterm infants, and the InterGrowth reference ranges are all used.[4] This limits the comparability of microcephaly prevalence estimates and accounts for a proportion of the global variability in rates. The WHO currently recommends the Intergrowth-21 criteria if the gestational age is known and the WHO Child Growth Standards if it is not.[1,5,6] In addition, and probably more importantly, inconsistency in frequency and rigour of measurement, and completeness of reporting, makes population prevalences difficult to ascertain. A recent study across Europe found a prevalence, excluding genetic conditions, of 1·53 (95% CI 1·16, 1·96) per 10,000 "births" (including live births, fetal deaths from 20 weeks gestation and termination of pregnancy for fetal anomaly). Including genetic conditions would increase the prevalence to approximately 2·0 per 10,000. There was considerable variation in the estimates from 0·41/10,000 in Portugal to 4·25/10,000 in UK and in the definition of microcephaly (<-2 *z*-scores, <-3 *z*-scores and clinical decisions), the reference ranges used and methods of ascertainment.[7]

Microcephaly can be divided into primary- i.e. develops before 32 weeks' gestation[8] or birth[9]- and secondary- i.e. microcephaly that develops after this time. Primary microcephaly, the focus of this review, is generally due to disturbed neurogenesis (mitosis or progenitor cell function) or death of neuronal progenitors.[9,10] Secondary microcephaly normally relates to the postnatal development and maturation of neurons (dendritic processes and synaptic connections).[8] Infection can lead to both primary or secondary microcephaly but this differentiation is often not possible if the appropriate head circumference measurements are not taken. In high-income countries,

microcephaly is predominantly due to non-infectious causes,[11,12] including genetic abnormalities, nutritional deficiencies, hypothyroidism, brain injury, alcohol, drugs, and placental insufficiency.[8,9] A multi-centre retrospective analysis from Germany found that only 25 of 403 (6·2%) cases of microcephaly (for which a cause was identified) were due to maternal infections in pregnancy.[12] The prevalence of in utero or perinatal infections in cases of severe microcephaly is higher e.g. 46 of 284 (16%) cases in New York 2013-15.[13]

A reduction in head size may be associated with intrauterine growth restriction, for example with CMV and ZIKV. This can be either symmetrically or asymmetrically reduced in size in relation to the overall anthropometry of the infant. The gestation at which an insult occurs, will determine when microcephaly is clinically detectable. An infection close to birth may result in brain damage but with a normal head circumference. Recent data suggest that microcephaly may be poorly sensitive for screening infants severely affected by congenital ZIKV infection, particularly as this consists of many other manifestations.[14] Clinically apparent, microcephaly is one of many fetal brain abnormalities that can lead to later neurodevelopmental sequelae.[15] Although a clinical diagnosis of microcephaly with normal brain imaging does not necessarily mean impaired brain growth, a reduction in skull volume is indicative of underlying cerebral cortical volume loss.[8,16] A study of Finnish infants admitted to a neonatal ward for example, showed that a one standard deviation increase in head circumference at birth was associated with a 0·8-1·5 unit increase in cognitive scores at 56 months.[17]

If we use microcephaly to screen for congenital diseases, and if OFC is normally distributed in the reference population, 2·3% of the population will be classified as having microcephaly, many of whom will be healthy. Conversely, many neonates with congenital infections that affect the brain will not have severe enough manifestations to cause microcephaly. To illustrate this, we simulate two scenarios (Figure 1). A definition of <-3 *z*-scores (0·1% of the population)[18,19] is

expected to be 99·9% specific, although much less sensitive (57%).[4] An assessment of 1501 cases of suspected microcephaly reported to the Brazilian notification system, showed that 21·7% of notified cases classified as 'definite or probable' had a normal head circumference (>-2 *z*-scores) and this cut-off had a sensitivity of 83% and specificity of 98%.[14] So although microcephaly is a good screening diagnosis for brain damage caused by a congenital infection, some cases will be normal and cases with neurological damage may not be identified. Further tools must therefore be employed.

Pathogenesis and embryology

The central nervous system becomes established from day 22 of embryonic development when the neural tube is formed.[20] The embryonic brain is initially composed entirely of proliferative neuronal progenitors which reside within the ventricular zone (VZ) bordering the neural tube lumen. However, with subsequent development, neurons begin to emerge and a new population of deeper subventricular zone (SVZ) neural progenitors arises. Proliferation of the SVZ cells contributes to further expansion of the brain's neuronal population. Proliferative VZ and SVZ neuronal progenitors persist until around mid-gestation, providing a target for pathogens via the cerebral blood supply which is now fully established. Experimental evidence shows that regulation of neural progenitor numbers and sub-types is vital for controlling brain size and morphology (Figure 2). For example, mouse studies reveal that abnormal expansion of the VZ or SVZ neural progenitors increases brain size and produces macrocephaly.[21,22] In contrast, a reduction in neural progenitor numbers due to cell death, cell cycle arrest or premature neuronal differentiation reduces brain size and produces microcephaly.[23-26]

The pathogenesis of CMV, HSV, rubella and ZIKV is described below. Little information is

available on the mechanisms of congenital brain infection for *T. gondii*, where additional research is needed. The congenital infections we describe in this paper (summarised in Table 1) are responsible for a range of developmental brain defects, with the more severe cases occurring after infection during the first trimester of gestation, when the neural progenitors are actively multiplying and producing neurons (Figure 2).[27-36]Analysis of material from aborted human fetuses confirms that brain cells are susceptible to viral infection (ZIKV,[30] CMV,[37] HSV[35,38] and rubella[39]). Moreover, human cell culture systems demonstrate that neural progenitors are targeted by these pathogens (ZIKV brain organoids[40,41]; HSV iPS-derived cell culture[42]; ZIKV neurospheres [43]; CMV neural precursors[37]). This research suggests that perturbation of neural progenitor populations may be the main cause of infection-related microcephaly.

CMV

CMV is capable of altering progenitor and neuronal fates through the downregulation of multipotency markers including Sox2 and Nestin.[44,45] Neuronal differentiation has been found to be inhibited or delayed by CMV[37,46,47] or to occur prematurely after infection.[44] Recently, a new molecular mechanism downstream of CMV brain infection has emerged: PPARγ (peroxisome proliferator-activated receptor gamma) was found to increase following CMV infection of human neural stem cells and human fetal brain sections. Activation of PPARγ function alone was sufficient to impair neuronal differentiation, and a PPARγ inhibitor restored normal differentiation. Moreover, nuclear PPARγ was detected in the brains of congenitally infected fetuses.[47] These findings support a role for PPARγ in mediating congenital CMV brain disease.

HSV

HSV can infect multiple brain cell types (human[35,38], in vitro [42] and mouse[48]) but how this leads

to microcephaly is unclear. Infected organs including the brain, show tissue necrosis post-mortem.[36] In vitro and animal studies suggest that HSV may initially induce an immune response that stimulates neural stem cell proliferation. Subsequently, brain infiltration by CD8(+) T cells limits proliferation through the stimulation of interferon-gamma.[49] Operation of a similar immune-mediated mechanism following congenital brain infection leading to microcephaly is possible but remains to be verified.

Rubella

The mechanisms by which rubella virus induces microcephaly remain largely unknown. Most human embryonic tissues can be infected by rubella virus[39,50] and brain vessels have been found to degenerate following rubella infection.[50-52] This suggests that a neurodegenerative mechanism could be a potential underlying cause of rubella-induced microcephaly in humans. There is also indirect evidence that rubella infection can slow the rate of cell division although this has not been confirmed in human neural cells in culture.[28]

ZIKV

Congenital viral infections and brain development studies have benefited from recent advances in the growth of human cells in 3-dimensional (3D) culture. Cell aggregates called ‘neurospheres’ are formed initially but with continued culture they can form ‘organoids’ that mimic some features of true organs. Studies employing these techniques have confirmed that neural progenitors (ventricular zone (VZ) and later subventricular zone (SVZ)) are indeed the commonest brain cell type infected by ZIKV (brain organoids[40,41] and neurospheres[43]). The exposure of these 3D cell cultures to the virus mimics many features of microcephaly in humans, including a decrease in neuronal production, reduced VZ thickness and overall smaller

organoids.[40,41,53] This reduction in growth seems to result from cell cycle arrest[54] and/or an increase in cell death,[41,43,53,54] and could indeed explain the microcephaly phenotype observed in ZIKV-infected human fetuses. Entry of ZIKV into neural progenitors has been suggested to occur via viral receptor AXL, which mediates ZIKV and dengue virus entry into human skin cells,[55] and is strongly expressed in VZ and SVZ neural progenitors.[56,57] However, a recent study using human brain organoids revealed that genetic ablation of AXL alone is unable to prevent ZIKV infection, suggesting that other cell adhesion or entry factors may be involved.[58] Human fetal organotypic slice culture studies reveal that phospho-TANK Binding Kinase 1 (pTKB1), relocates from centrosomes to mitochondria following ZIKV infection producing mitotic defects and supernumerary centrosomes that may exacerbate cell death.[57,59] Injection of ZIKV into pregnant macaques and mice, or into the mouse brain, has recently confirmed some of the observations from human in vitro culture systems.[60-62] Dang and colleagues also discovered that ZIKV infection activates TLR3 (involved in activation of an immune response) and that TLR3 inhibition can attenuate apoptosis and decrease growth induced by viral infection.[40] The comparison of transcriptome profiles derived from infected and non-infected animal tissue also highlights new potential molecular viral targets. These include the down-regulated expression of an extensive list of genes (all centrosome-related) previously associated with autosomal recessive primary microcephaly (ASPM, CENPF, MCPH1, STIL, Cep135).[60,61] This suggests that the underlying pathogenesis of autosomal recessive and virally induced microcephalies may be similar, although this is yet to be confirmed.

Infection in pregnancy: Epidemiology, transmission and clinical features

Table 1 describes the epidemiology and clinical features of the main infections associated with microcephaly in pregnant women/adults.

CMV

Most women of childbearing age in low- and middle-income country (LMICs) have long-lasting immunity from prior CMV infection but viral reactivation can take place in pregnancy or during periods of immunosuppression. In Sub-Saharan Africa, Latin America and South Asia, over 90% of the general population have IgG antibodies compared to a seroprevalence of 40-60% in high-income countries.[63-68] Prevalence of congenital CMV infection in high-income countries is estimated to be 0·7% of all live births, or 1-5% in low-income countries.[63,66,69]

The risk of *in utero* transmission varies according to whether maternal CMV infection is primary, a reactivation of latent infection, or superinfection with another CMV strain.[63] Primary infection has the highest risk of in-utero transmission and fetal disease is more severe, particularly when it occurs earlier in pregnancy. Overall, most cases of congenital CMV infection result from non-primary maternal infection but most of these are asymptomatic.[70] The risk of transmission increases with advancing gestational age, with 35% of mothers who have a primary infection in the first trimester giving birth to infected newborns, compared to 65% who are infected in the third trimester.[71] Rates of transmission in non-primary infection are less certain but are generally reported to be lower.[63,72,73] The risk of congenital CMV infection is increased by maternal HIV infection both for HIV exposed uninfected and HIV infected infants.[63] In-utero HIV transmission has been shown to be particularly associated with congenital CMV.[74]

HSV

Primary microcephaly is a relatively rare complication of perinatal HSV infection and is mainly found in association with in-utero infection which accounts for only 5% of cases.[75] Global

average seroprevalence is 18% amongst women of childbearing age but this includes huge variations: from 4·1% in Japan to 62% in East Asia and 70% in Sub-Saharan Africa.[76,77] Neonatal infection at the time of birth from mothers with symptomatic genital infection is more common but does not cause primary microcephaly.

Rubella

Rubella is a vaccine-preventable disease and rates of infection are largely dependent on the coverage of immunisation programmes. The global incidence of congenital rubella syndrome has reduced from 0·1-0·2 per 1000 live births prior to vaccination to near elimination (<0·01 per 100,000 live births) in areas with comprehensive vaccination rates.[78] Vaccination programmes were widespread in most countries in 2014 except in Sub-Saharan Africa and South Asia.[79] Despite widespread vaccination programmes an estimated 9.4% of pregnant women remain seronegative worldwide and therefore susceptible to infection.[80] In areas without vaccination programmes, incidence of congenital rubella syndrome has been estimated at 19-283 per 100,000 live births in the African region and 18-309 per 100,000 live births in the South Asian region.[78]

T. gondii

Most women of childbearing age in Latin America (51-72%), Central Europe (58%), and West Africa (54-77%) have specific IgG antibodies to *T. gondii*.[81] Conversely, relatively few women of childbearing age are found to be seropositive in South-east Asia, China and Korea (4-39%), Scandinavia (11-28%), and the United States (15%).[81,82] Mother-to-child transmission predominantly occurs following primary infection during pregnancy. The global incidence of congenital toxoplasmosis is estimated at 1·5 cases per 1000 live births (95% credible interval 1·4, 1·6), being highest in some Latin America countries (average, 3·4 per 1000) and lowest in parts

of Europe (0·5 per 1000).[83] The risk of congenital infection may rise due to reactivation of *T. gondii* from immunosuppression, for example resulting from HIV.[84]

ZIKV

ZIKV has been described for over 60 years with intermittent case reports until outbreaks in Yap Island (estimated 73% (95% CI 68, 77) of population infected) in 2007, French Polynesia (up to 66% IgG +ve) in 2013 and Brazil in 2015.[55,85,86] It is currently documented in 84 countries or subregional areas and 31 have reported microcephaly or neurological malformations associated with ZIKV.[87] The prevalence of suspected disease in women of childbearing age is 5400 per 100,000 in Brazil.[88]

Laboratory diagnosis during pregnancy

Any pregnant woman presenting with fever and/or rash should be evaluated for risk of infections that are potentially transmitted to the fetus. There are many infectious causes of rash, for example Parvovirus B19, measles and varicella zoster, and extensive guidelines exist describing when testing should be done.[89]

During the acute infection, laboratory confirmation is usually only possible where clinical symptoms are present in the mother. Primary CMV, HSV, *T. gondii*, and ZIKV infections may be asymptomatic and therefore the opportunity to confirm acute infection can be missed.[90] In acute symptomatic maternal infection, ZIKV and rubella can be detected in serum, blood, oral fluid or urine by PCR amplification of nucleic acid and may precede the development of IgM antibodies. Acute primary maternal HSV can be diagnosed by viral PCR and the presence of IgM antibodies but usually only when oral or genital lesions are present. Virus may be detected for up 10 weeks in serum following acute ZIKV[29] and two weeks following acute rubella[91] while CMV and HSV

are frequently shed asymptomatically from mucosal epithelia.[92,93] Detection of *T. gondii* IgA is the most sensitive indicator of congenital infection in the child.[94] CMV shedding in oral fluid may occur both during primary infection and asymptomatic reactivation and is therefore not always a reliable indicator of acute primary infection. Detection of virus needs to be interpreted in the light of serological results.[66]

Serological testing in pregnant women

For rubella, *T. gondii* and CMV commercial enzyme immunoassays are routinely available for antibody detection. For rubella and toxoplasmosis cases, serological diagnosis of acute primary infection relies on detection of acute phase IgM antibodies, which for most do not reach adequate levels until one week following acute infection. For all three, paired IgG samples taken at least 10-14 days apart should also be obtained to confirm seroconversion. A fourfold rise in antibody titre is indicative of recent although not necessarily primary infection.[94] *T. gondii* IgM antibodies are commonly detected by ELISA in acute infections, but may persist for several months.[95] High levels of IgG antibodies may also be present during the acute phase of infection.[96]

In general, detection of CMV IgM, has been shown to lack sensitivity and specificity, especially in conditions of immune dysfunction. A positive CMV IgM is associated with primary infection in only 10% of cases. To distinguish between acute and chronic infections, ELISA-based IgG avidity tests are widely recommended: low-avidity antibodies are typically found over the first weeks, while high-avidity IgG predominates in the chronic phase.[93]

IgM and IgG antibody tests for dengue, West Nile fever and ZIKV members of the flaviviridae family share considerable epitope cross-reactivity. Plaque reduction neutralising tests can be used to differentiate closely related viruses but is too complex for routine diagnosis especially in non-specialised laboratories.

In-utero testing

In the event that acute maternal infection with any of the above pathogens is considered to pose a risk, infection of the infant can be investigated where possible by amplification of pathogen nucleic acid from amniotic fluid. Amniotic fluid samples are typically obtained at 18 weeks gestation to determine fetal infection with *T. gondii* and guide therapy.[97] In CMV infection, there is a 6-8 week window between maternal infection and being able to detect the virus in amniotic fluid. PCR has high sensitivity when performed at the correct time (20-21 weeks gestation or 7 weeks after maternal infection) and, when combined with culture, nearly all congenital infection can be diagnosed.[63] PCR testing of amniotic fluid for HSV is possible but does not seem to correlate with neonatal infection,[98] and has recently been shown to be possible with ZIKV.[99]

Screening

Childhood immunisation programmes against rubella are routinely available in 147 countries, with an estimated global coverage of 46%.[100] Pregnant women may be screened for rubella antibody and those in whom levels are absent or low, offered post-partum vaccination. Recently, high coverage levels of immunisation have led to some high-income countries to drop rubella screening. Prenatal screening for toxoplasmosis is routinely undertaken in Austria, France and Slovenia and neonatal screening in Denmark, Ireland and some parts of USA and Brazil.[95] In France, women who develop high titres of IgM antibodies or evidence of seroconversion indicative of primary infection during pregnancy are offered fetal screening and treatment with spiramycin or pyrimethamine-sulphonamide. Severe sequelae from congenital toxoplasmosis are now rarely seen after the current approach of systematic prenatal screening and treatment was implemented in France.[101] Screening for CMV in some high-income countries and for ZIKV in

countries with high levels of transmission are being considered however, it is not currently recommended for either.

Clinical presentation at birth

Whilst infection can cause both primary and secondary microcephaly, the main focus of this review is the effect of congenital infections on neurogenesis or antenatal neural progenitor death usually associated with primary microcephaly, i.e. it is present at birth. Evaluation of an infant with suspected microcephaly at birth seeks to meet three goals: (1) confirm the diagnosis of microcephaly; (2) identify the cause and attempt syndromic diagnosis; and (3) aid prognosis and guide initial treatment where appropriate.

Measurement of the OFC (illustrated in Figure 3) is used as a screening examination to identify neonates who are likely to have an underlying neurological condition. A full antenatal history with particular focus on acquired environmental insults, maternal serology, antenatal fetal growth measurements where available, and family history of microcephaly and neurological conditions are essential to identify possible causes. Systemic examination, including ophthalmology and audiology, will help in identifying associated abnormalities in other organ systems. Cranial ultrasound (crUS) can be used to screen for anatomical abnormalities where available. Features suggestive of congenital infections in an infant with microcephaly are: maternal history of infection during pregnancy, indicative antenatal maternal serology results, clinical features in the neonate (Panel) and calcifications on brain imaging. In patients with these features, a more specific work-up is required, including serological testing to confirm specific infectious causes, other laboratory investigations, and further neuroimaging.

Identifying Specific Infectious Causes

Common clinical features for each infection are shown in the appendix and a summary of diagnosis and treatment recommendations are given in Table 2. Much of this information is based on historical cohorts and case studies and is therefore potentially prone to bias. Individual patients may present with different signs and symptoms.

CMV

Most neonates with congenital CMV are asymptomatic but common manifestations in symptomatic neonates include intra-uterine growth restriction, sensorineural hearing loss, petechiae, and jaundice. Neurological sequelae are observed in 60-90% of those with clinical symptoms at birth,[102] although biases from early cohort studies may overestimate this.[72,103,104] Up to 15% of infants that are asymptomatic at birth may go on to develop symptoms later in childhood.[63,102,104]

HSV

Infection can occur *in utero*, intrapartum or postnatally. *In utero* infection is classically associated with a triad of cutaneous, ophthalmological and neurological abnormalities (including microcephaly). Vesicular lesions may or may not be present and often develop late. Perinatal infection presents in 3 main ways: disseminated disease affecting multiple sites, CNS disease or skin/eyes/mouth limited infection.[75,105]

Rubella

Fetal abnormalities resulting from *in utero* transmission of rubella range in severity according to the gestation at the time of infection. Infection in the first trimester is associated with more severe abnormalities.[106] Symptomatic infection in the infant is referred to as congenital rubella syndrome

(CRS) with a classic triad of cataracts, sensorineural deafness and cardiac defect (e.g. patent ductus arteriosus, ventricular septal defect).[107] Estimates of the frequency of microcephaly in CRS vary but have been reported to be as high as a third of cases overall.[108]

T. gondii

Approximately 24% of live born infants infected with *T. gondii* are symptomatic at birth.[109] The classic signs originally described by Sabin (chorioretinitis, microcephaly or hydrocephalus, and widespread intracranial calcifications) are relatively infrequent, but highly suggestive of the diagnosis of congenital toxoplasmosis. More severe manifestations occur in infections earlier in gestation. Intracranial lesions, for example, are seen in up to 40% of congenital infections acquired before 5 weeks of pregnancy but in less than 10% of those acquired beyond 20 weeks.[109] 12·5% of liveborn infants with congenital toxoplasmosis develop severe neurological sequelae;[110] 5% of them have microcephaly.[111]

ZIKV

ZIKV infection in pregnancy and its possible link to a range of birth defects is currently the subject of intense investigation worldwide. There is now convincing evidence that ZIKV infection in pregnancy, especially in the first trimester, is associated with an increased risk of microcephaly. The risk of microcephaly associated with ZIKV infection was estimated as 95 per 10,000 infected women in first trimester in French Polynesia.[112] Congenital disease has predominantly been seen in Brazil, but this may spread across Latin America. Brasil et al followed up ZIKV affected pregnant women in Rio de Janeiro and found that 3.4% had microcephaly and 42% had abnormal clinical or radiological findings in the first month, mostly affecting the central nervous system.[113] The spectrum of congenital Zika syndrome (CZS)

includes birth defects associated with microcephaly (including hearing loss and ophthalmological defects) and how they are related to timing of infection, and presence or absence of maternal symptoms, as well as the influence of other arboviral infections has yet to be fully delineated. Currently, most of what is known about CZS comes from neonates with microcephaly which appears to include syndrome-specific features such as partially collapsed skull, reduced cortical thickness and extensive subcortical calcifications.[114,115] However, the full spectrum will only become clear when cohorts of infected mothers provide in-depth report of the clinical presentation. Brain damage or ocular lesions can be found in the absence of microcephaly and other features, as described in the appendix, are common.[14,116,117] It is yet to be shown whether there is ongoing viral replication at the time of birth in congenital ZIKV infection. This will have important implications for the potential efficacy of postnatally administered antiviral therapy.

Markers for Prognosis

Microcephaly outcomes are varied, and more accurate prognosis at the time of diagnosis is one of the main aims of clinical evaluation of the neonate. Neuroimaging- for example crUS, CT and MRI- has proven a useful predictive investigation and is warranted in neonates with severe microcephaly, or where a congenital infection is suspected.[118] Whilst different modalities have specific advantages (e.g. good visibility of calcifications on CT, bedside availability of crUS), many structural abnormalities caused by disruptions in development can be identified across modalities. Typical abnormalities include intracranial calcifications, white matter abnormalities (e.g. periventricular leukomalacia, delayed myelination; best seen on MRI), gyration defects (e.g. polymicrogyria), and schizencephaly.[12,119]

Long-term consequences and resulting disability in childhood

Long-term follow-up is recommended for infants with microcephaly and even for those with congenital infections and who are apparently unaffected at birth in order to manage evolving conditions and identify new manifestations early. In many cases, the cause of microcephaly will not be known and generic plans can be adopted to manage impairments and limit disability. The timing of the interventions vary in importance. Hearing screening for example needs to be done early to enable interventions to optimise language development. Other interventions, including psychosocial support and counselling,[120] are required throughout childhood and into adult life. Where microcephaly occurs, recommendations are for early intervention to address associated impairments, calling upon a host of specialist medical and educational services.[121] Currently, regular follow-up is recommended for ZIKV associated microcephaly for anthropometry, developmental and neurological assessments and also hearing and ophthalmological assessment where required over the first two years, with less frequent follow-up recommended for infants without microcephaly.[1] Evidence-based therapy strategies exist that may improve cognitive outcomes and reduce disability.[122] Interventions in early life, for example child stimulation,[123] maximise the plasticity of the developing brain, attenuating the consequences of the damage to the nervous system. But such recommendations ignore the realities of life in many countries. Issues begin at birth, for example, in some societies, children with visible disabilities are allowed to die in the neonatal period, either through active infanticide or through withdrawal of basic care such as feeding.[124]

The provision of both recommended early diagnosis, and appropriate interventions may be severely limited in countries with only a handful of specialists, where early intervention programmes or inclusive education efforts remain rare and are largely urban-based. Based on a small but growing literature on disability, individuals with more severe disabilities are far less likely to receive appropriate medical care, attend school or are included in the social, economic or

religious life of their communities.[125] This is compounded in poorer households, which often choose to invest limited resources on non-disabled children, whom they feel will be able to contribute to the household in future.[126] Furthermore, recommendations for individuals with microcephaly tend to concentrate on early childhood, but anticipate little about their management, support or advocacy needs beyond pre-school years. While attitudes vary from society to society, overwhelmingly, persons with disabilities face increased risk of stigma, social isolation, abuse and poverty across their lifespan.[127]

Infections that cause microcephaly are likely to disproportionately affect LMICs and within these countries, the poorest populations, who are more likely to live in crowded areas with inadequate housing and limited water and sanitation systems. Compounding this, poorer women are less likely to have access to family planning services, to access prenatal screening or, if needed, safe termination of pregnancy.[128]

Although social protection schemes are beginning in many middle-income countries,[129] the cost of raising and supporting a disabled child continues to be borne almost entirely by the immediate family. Households with disabled members are on average, poorer because of increased costs and because family members, in particular women, must take time away from income generating activities to provide care.[130]

Improved services and support for affected individuals and families across the lifespan must be anticipated, and health professionals must work with civil society organisations, including disabled peoples organisations, to ensure these children and their families receive the medical, educational and social service support they need and are entitled to.[131]

Conclusions

We have summarised the epidemiology, clinical presentation and the current understanding of the

pathogenesis of the major congenital infections associated with microcephaly. Potential inconsistencies in the criteria used to diagnose microcephaly have been highlighted as well as limitations of current diagnostic methods in confirming an infectious aetiology. If microcephaly, as strictly defined by head circumference, is used as a screening tool, it will miss affected infants who do not manifest with a small head size. Many infants are likely to have subtle deficits needing more sensitive neurological and developmental assessments. Microcephaly is also usually only one manifestation in a syndrome and other assessments are required to identify the full spectrum of illness.

ZIKV has brought the attention of the world to the problem of microcephaly. The extent of the disease burden resulting from ZIKV is still being clarified but it appears to be significant and is one of a number of infections associated with primary microcephaly. On an individual level, accurate diagnosis and thorough investigation of confirmed microcephaly can guide management and determine prognosis. On a population level, assessing the overall burden and contribution of different causes will guide future interventions and strategies for prevention. Preventing infection, where possible, must be prioritized. It is not within the scope of this review to fully consider health protection and primary prevention but key interventions exist, for example population immunisation for rubella and vector control for ZIKV. Pharmacological interventions are able to treat some congenital infections and therefore limit further damage to the child's developing nervous system (CMV, HSV, *T.gondii*). Antiviral treatments are not currently available to treat ZIKV in pregnancy but vaccines are in development. Interventions to attenuate the effects of the developmental delay, and at the community or population level to minimize impairment caused by infections that can lead to microcephaly are all needed. More clinical and health system research is crucial to provide scalable interventions to reduce the disability associated with microcephaly. Many interventions designed to optimise neurodevelopmental outcomes in

infected infants will be common to all congenital infections and enhanced services for ZIKV follow up could potentially also be used in the future for the benefit of children severely affected by other congenital infections.

Regions in which the infective burden is highest are also likely to be those where diagnosis and management is most lacking, creating a disproportionately large burden on populations that can least afford it. Inequities in access to optimised multidisciplinary care for children with neurodisability in areas with the highest rates of microcephaly means that the prognosis for those infants is often very poor. ZIKV has highlighted the issue of microcephaly and provides a timely opportunity to refocus on the whole group of infectious diseases which can affect the developing fetal brain and to redouble our efforts for prevention and treatment in the short- and long-term for those living with the frequently devastating consequences of such congenital infections.

Panel: Clinical features suggestive of congenital infection in a neonate with microcephaly.[132,133]

- Severe microcephaly (<-3 *z*-scores)
- intrauterine growth restriction
- hydrops fetalis
- seizures
- cataract and other visual abnormalities
- hearing loss
- congenital heart disease
- hepatosplenomegaly
- jaundice
- characteristic rashes

Table 1. Summary of the microbiological, virological and maternal clinical features

Organism	**Description**	**Transmission**	**Risk period for transmission of infection to the fetus**	**Maternal signs and symptoms**	**Prevention of infection and treatment of pregnant women**
CMV	Betaherpes DNA virus *Herpesviridae* family	Infectious body fluid (saliva, urine, breast milk, genital secretions, blood transfusion).	Risk increases with gestational age, but earlier infection is associated with more severe congenital infection.	Mostly asymptomatic or flu-like symptoms	Treatment with antiviral medication not currently recommended outside of trial settings. Candidate vaccines are in development. Hyperimmune globulin has been trialled but has not as yet been shown to reduce transmission of CMV to the fetus.[134]
HSV 1 & 2	Alphaherpes DNA viruses. *Herpesviridae* family	Orogenital, genital-genital, transmitted during delivery through infected maternal genital tract. Virus shed with or without	Risk is associated predominantly with primary infection late in pregnancy.	Primary genital herpes infection may be asymptomatic but is often associated with mucocutaneous lesions.[75]	Risk of perinatal transmission can be reduced by Caesarean section in the context of active genital lesions and suppressive

		lesions present.		Latent infection is subsequently established in dorsal root ganglia. Mucocutaneous lesions can then recur following viral reactivation.[105]	aciclovir/valaciclovir given to the mother prior to delivery.[135]
Rubella virus	Single stranded RNA virus *Togaviridae* family	Respiratory transmission. Humans are the only known host of rubella.[136]	Risk of congenital disease in first 20 weeks; infection beyond this not associated with congenital defects. Primary maternal infection is associated with up to a 50% risk of fetal infection.[137]	Typically a mild self-limiting illness Maculopapular rash, lymphadenopathy, malaise, arthralgia, fever.[136]	Immunisation of girls to prevent individual cases and the whole population to interrupt transmission. Supportive management of the infection.
T. gondii	Obligate intracellular protozoan parasite	Humans acquire infection mainly by consuming raw or undercooked meat containing cysts, by ingesting water or raw vegetables contaminated with oocysts, or by	Risk of congenital transmission increases with gestational age at maternal infection: 15% (95% CI 13–17) at 13 weeks and 71% (95% CI 66-76) at 36	Most acute *T. gondii* infections in immunocompetent pregnant women are subclinical; fever, lymphadenopathy and a	Treatment with spiramycin and/or pyrimethamine-sulphonamide of maternal toxoplasmosis diagnosed through prenatal screening is believed to reduce the risk of

		transplacental transfer of tachyzoites during an acute infection. Tachyzoites invade host cells, multiply and disseminate, infecting multiple sites in the body including brain, eye, heart, skeletal muscle and placenta.[138] Toxoplasmosis reactivation in immunocompromised women may rarely be a cause. Blood transfusion or organ transplantation. The only definitive hosts are members of the cat family.[138]	weeks.[109]	flu-like illness are the most common clinical signs and symptoms.[138]	mother-to-child transmission and neurologic sequelae in the fetus in settings where low-virulence type II strains predominate, but this protective effect remains to be confirmed against more virulent atypical recombinant strains.[101]
ZIKV (much knowledge still	Single stranded RNA arbovirus *Flaviridae*	The main vector is the *Aedes* mosquito. *A. aegypti* is considered of greatest global importance.[139]	Risk of transmission appears to be throughout pregnancy	Infection is often asymptomatic in adults with symptoms reported in approximately 20% of	Primary prevention of mosquito bites. No treatment as yet.

provisional)	family There are currently two major lineages: one African (with two groups, the Uganda cluster and the Nigeria cluster) and one Asian/American.	Also sexual transmission and potentially through infected blood transfusion.[140] Non-human hosts are thought mainly to include non-human primates, although evidence of infection has also been demonstrated in other mammals.[139]		cases.[141] Common features include pruritic, maculopapular rash, low grade fever, arthritis/arthralgia, conjunctivitis, myalgia and headache. Has been associated with Guillain-Barré syndrome, myelitis and meningoencephalitis reported in adults.[142]	

Table 2: Diagnosis and treatment of infants

Organism	**Diagnosis**	**Treatment**
CMV	CMV viral culture or PCR testing for DNA from urine or saliva obtained within the first three weeks of life. Retrospective diagnosis can be achieved from dried blood samples taken for newborn screening. Testing should be done as early as possible.	Early treatment of confirmed symptomatic congenital CMV with IV ganciclovir or oral valganciclovir is currently recommended based on the results of 2 main trials.[143-145] Results from randomised trials and observational studies have demonstrated improved hearing and

		neurodevelopmental outcomes although questions remain regarding optimal treatment strategies, including when to treat and duration of therapy.
HSV 1 & 2	PCR testing for viral DNA on surface swabs (conjunctivae, mucosal surfaces), cutaneous lesions, CSF, whole blood, and tracheal secretions where available.	Intravenous high-dose acyclovir is indicated for confirmed neonatal HSV infections.[146]
Rubella	Isolation of rubella virus (or viral RNA) from the neonate, isolation of rubella IgM or persistent rubella IgG. The virus is most commonly isolated from nasopharyngeal samples, but can be from blood, urine, and CSF cultures.[147]	Management is mainly supportive and involves monitoring for late emerging symptoms (e.g. hearing loss, endocrine problems).
T. gondii	Diagnosis is difficult if specific IgM and IgA antibodies are not detected in the serum or plasma at birth. CSF samples to detect IgM and IgA antibodies may confirm the diagnosis in infants with intracerebral lesions. Maternal IgG is detectable in the fetus for several months, generally disappearing completely within one year. Because specific IgG produced by congenitally infected newborns may be detected about 12 weeks after birth, the presence of high IgG titres beyond this time is suggestive of congenital infection.[95]	Congenital toxoplasmosis is treated with pyrimethamine and sulphonamide (sulphadiazine or sulphadoxine) with folinic acid to minimize pyrimethamine-associated hematologic toxicity. Up to 85% of children with subclinical congenital infection, if left untreated, will later develop signs and symptoms of disease, such as chorioretinitis or developmental delays.[148]

ZIKV	Testing for IgM antibody with capture ELISA using recombinant antigens in CSF or blood at birth indicate congenital infection; plaque reduction neutralisation tests are required to confirm monotypic antibody responses, although dengue and yellow fever do not cause congenital infections. Reverse-transcriptase PCR detects acute infections, positivity rare in neonates. Tests can be done on serum/plasma from cord blood or infant's peripheral blood. IgM detection in CSF of neonate is highly specific.	Clinical management of complications, including dysphagia, irritability and epilepsy. Supportive care for neurocognitive delays, hearing and visual loss, and appropriate developmental follow-up.

CNS = central nervous system, CSF = cerebrospinal fluid, PCR = polymerase chain reaction

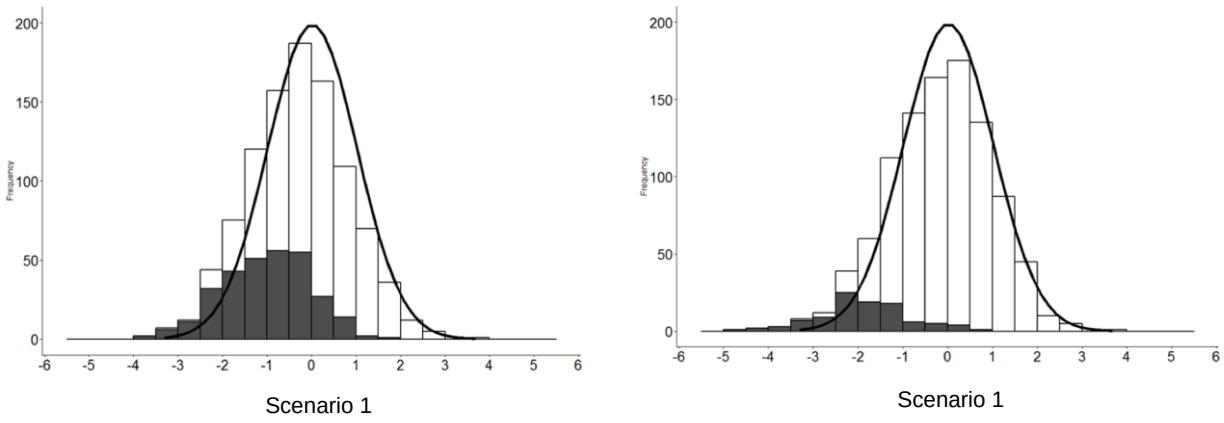

Figure 1: Modelled population effects of insults to brain developmental

* Bars represent the OFC distribution at birth (*z*-scores on *x* axis). The dark bar segments indicate the head circumference measurements of these "affected" children. The continuous line represents the reference population (no children "affected"), with a mean of 0 and SD of 1. Simulated populations were created using R language and environment for statistical computing.[149]

If an otherwise normal population of 1000 infants (in which children's occipito-frontal circumferences (OFCs) are close to their expected values) is exposed to a congenital infection that limits brain development, a proportion of children are labelled as "normal" by being above the -2 *z*-scores (standard deviations (SD)) or -3 *z*-scores cut-offs but they may deviate from their "expected" OFC.

Scenario 1 describes a congenital infection with a uniform shift to the left by 1 SD relative to the reference curve in 30% (randomly chosen) of affected children, roughly as expected in congenital toxoplasmosis acquired late in pregnancy. The remaining 70% children were not affected at all. In this hypothetical scenario, 6·5% of children fall below -2 *z*-score cut-off value for microcephaly; 0·9% below the -3 *z*-scores. Only 65 of the 300 (21·7%) of the "affected" children will actually be classified as "microcephalic" by using the -2 *z*-score cut-off. Only children whose "ideal" OFC would be less than -1 SD of the expected mean (expected proportion of 15·9% in a

healthy population), would actually be diagnosed as “microcephalic” after the 1 SD shift caused by the infection.

Scenario 2 is a congenital infection where OFC is shifted to the left by 2 SD in 10% of children, roughly as expected for congenital toxoplasmosis acquired early in pregnancy. In this hypothetical scenario, 6·5% of children fall below -2 *z*-score cut-off value for microcephaly; 1·4% below the -3 *z*-scores. Here, 65% of “affected” children will fall below the cut-off value of -2 SD.

By counting the number of children below a given cut-off value we are severely underestimating not only the proportion of children who have been relatively mildly affected (Scenario 1) but also that of children who have been more severely affected (Scenario 2). Moreover, although Scenarios 1 and 2 represent conditions varying in severity which affect different proportions of children in the population, in both examples the prevalence of microcephaly, estimated by applying the -2 *z*-score cut-off, would be quite similar (around 6.5%).

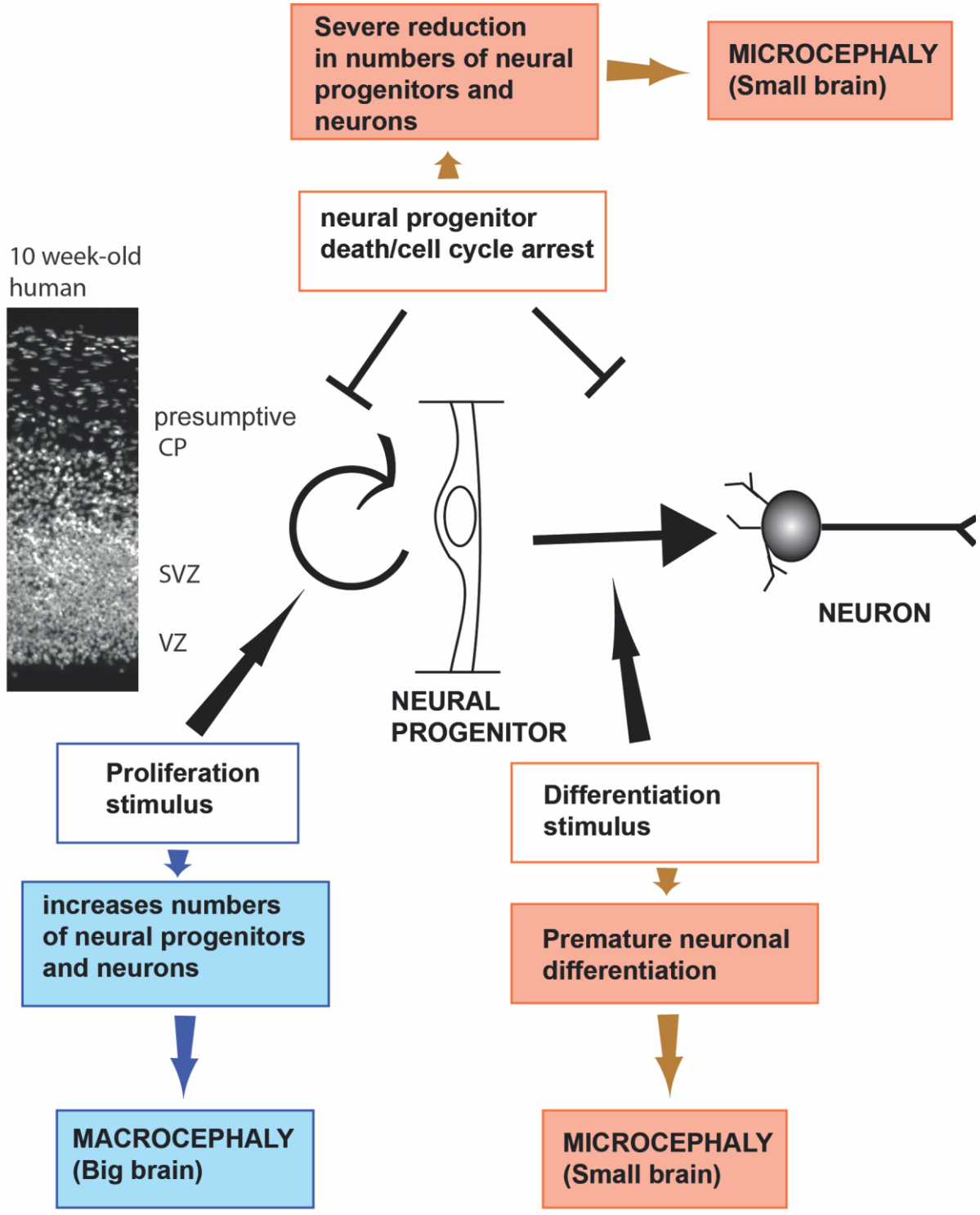

Figure 2. Factors that may influence neuronal and neural progenitor populations and lead to microcephaly or macrocephaly

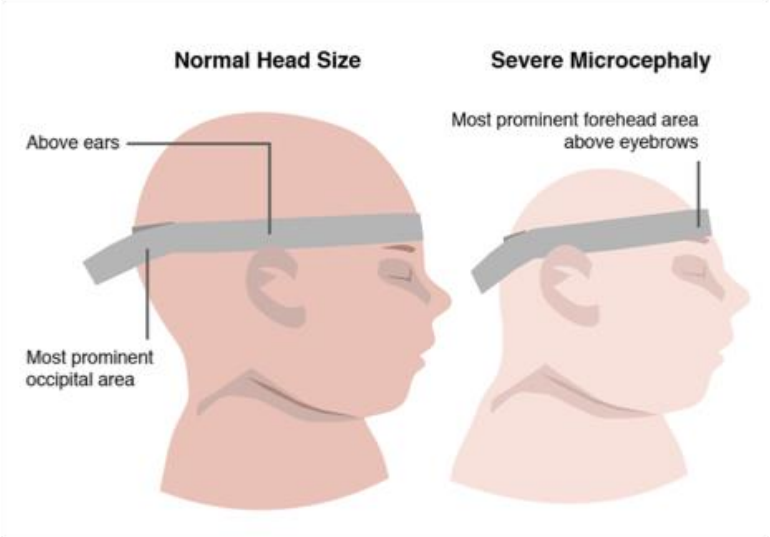

Figure 3: **Measurement of occipito-frontal circumference (OFC) in the neonate**

Measurements of OFC can be variable and user dependent. To aid robust measurements, a non-stretchable tape should be used and placed above the ears, covering the broadest part of the forehead, and the most prominent area of the occiput as shown. The largest measured circumference of three repeat measures should be used and recorded to the nearest millimeter. In neonates, the most robust measurements are achieved at >24h of age when post-partum skull modelling has subsided.

Search strategy

Pubmed, Embase and Google Scholar were searched, with no language or date restrictions (search end date: 31 March 2017), for the following terms and reference lists were searched for additional citations:

- (Cytomegalovirus or CMV or Rubella or MMR or Zika or HSV or herpes or toxoplasma or toxoplasmosis) and (seroprevalence or prevalence or incidence)

- (congenital pathogenic infections or pathogenic infections) and (brain disorders or microcephaly)
- (pathogens or virus or pathogen or viral) and microcephaly
- (Cytomegalovirus or CMV or Rubella or MMR or Zika or HSV or herpes or toxoplasma or toxoplasmosis) and (brain or brain disorder or brain development or brain defects)
- (cytomegalovirus or rubella or toxoplasmosis or herpes simplex or varicella or chikungunya or West Nile virus or HIV or syphilis) AND microcephaly NOT Zika

Conflicts of interest

We declare that we have no conflicts of interest.

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Author contributions

DD and IA conceived the work. AB, DD, JoB, MUF, PA and RR undertook the literature reviews. AB, AJC, DD, JoB, JuB, LCR, MAC, MUF, NEG, PA and RR wrote the sections of the draft. All authors interpreted and critically revised the draft.

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Ethics approval

Not applicable. This a review of published literature.

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