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1 **Infectious causes of microcephaly: Epidemiology, Pathogenesis, Diagnosis, and**  
2 **Management**

3 Devakumar D\*, Bamford A, Ferreira MU, Broad J, Rosch R, Groce N, Breuer J, Cardoso MA, Copp AJ,  
4 Alexandre P, Rodrigues LC, Abubakar I.

5 \*Corresponding author: Dr Delan Devakumar, UCL Institute for Global Health, 30 Guilford St,  
6 London. WC1N 1EH Tel: +44 (0)20 7905 2122 or +44 (0)7894 579082. [d.devakumar@ucl.ac.uk](mailto:d.devakumar@ucl.ac.uk)

7

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12

13 **Summary**

14 Microcephaly is an important sign of neurological malformation and predictor of future disability.

15 The recent outbreak of Zika virus and congenital Zika infection has brought the world's attention  
16 to the links between infection and microcephaly. However, Zika virus is only one of the

17 infectious causes of microcephaly and, though the contexts in which they occur vary greatly, all

18 are of concern. In this review, we summarise important aspects of the major congenital infections

19 that can cause microcephaly, describing the epidemiology, transmission, clinical features,

20 pathogenesis and long-term consequences. We include the infections that cause substantial

21 impairment: cytomegalovirus, herpes simplex virus, rubella virus, *Toxoplasma gondii*, and Zika

22 virus. We highlight potential issues with the classification of microcephaly and show how some

23 infants affected by congenital infection may be missed or incorrectly diagnosed. While the

24 world's current focus on Zika virus is remarkable, preventing all infectious causes of

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

27

## 28 **Introduction**

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

38 In this article, we review the major congenital infections that can cause microcephaly, focusing  
39 on those with the largest disease burden and the strongest evidence for causation:

40 cytomegalovirus (CMV), herpes simplex virus (HSV), rubella virus, *Toxoplasma gondii* (*T.*  
41 *gondii*), and ZIKV. We describe the epidemiology, pathogenesis, transmission, clinical features  
42 and long-term disability in childhood. Less frequent causes of primary microcephaly are not  
43 considered here. While congenital syphilis has a high disease burden in regions affected by ZIKV  
44 and varicella virus has recognised neurotropic properties, published reports describing primary  
45 microcephaly in the context of these two infections are scarce.

46

## 47 *Microcephaly*

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

50 An accepted definition of microcephaly is occipito-frontal head circumference (OFC) > 2 standard  
51 deviations below the median (< -2 z-scores), for gestational age and sex in an appropriate healthy  
52 reference population, with severe microcephaly defined as < -3 z-scores.<sup>1-3</sup> The reference range  
53 and population from which a definition is taken can vary, for example country-specific ranges,  
54 the World Health Organization (WHO) child-growth ranges for term infants, the Fenton ranges  
55 for preterm infants, and the InterGrowth reference ranges are all used.<sup>4</sup> This limits the  
56 comparability of microcephaly prevalence estimates and accounts for a proportion of the global  
57 variability in rates. The WHO currently recommends the Intergrowth-21 criteria if the gestational  
58 age is known and the WHO Child Growth Standards if it is not.<sup>1,5,6</sup> In addition, and probably  
59 more importantly, inconsistency in frequency and rigour of measurement, and completeness of  
60 reporting, makes population prevalences difficult to ascertain. A recent study across Europe  
61 found a prevalence, excluding genetic conditions, of 1.53 (95% CI 1.16, 1.96) per 10,000  
62 “births” (including live births, fetal deaths from 20 weeks gestation and termination of pregnancy  
63 for fetal anomaly). Including genetic conditions would increase the prevalence to approximately  
64 2.0 per 10,000. There was considerable variation in the estimates from 0.41/10,000 in Portugal to  
65 4.25/10,000 in UK and in the definition of microcephaly (< -2 z-scores, < -3 z-scores and clinical  
66 decisions), the reference ranges used and methods of ascertainment.<sup>7</sup>

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

74 microcephaly is predominantly due to non-infectious causes,<sup>11,12</sup> including genetic abnormalities,  
75 nutritional deficiencies, hypothyroidism, brain injury, alcohol, drugs, and placental  
76 insufficiency.<sup>8,9</sup> A multi-centre retrospective analysis from Germany found that only 25 of 403  
77 (6.2%) cases of microcephaly (for which a cause was identified) were due to maternal infections  
78 in pregnancy.<sup>12</sup> The prevalence of in utero or perinatal infections in cases of severe microcephaly  
79 is higher e.g. 46 of 284 (16%) cases in New York 2013-15.<sup>13</sup>

80 A reduction in head size may be associated with intrauterine growth restriction, for example with  
81 CMV and ZIKV. This can be either symmetrically or asymmetrically reduced in size in relation  
82 to the overall anthropometry of the infant. The gestation at which an insult occurs, will determine  
83 when microcephaly is clinically detectable. An infection close to birth may result in brain damage  
84 but with a normal head circumference. Recent data suggest that microcephaly may be poorly  
85 sensitive for screening infants severely affected by congenital ZIKV infection, particularly as this  
86 consists of many other manifestations.<sup>14</sup> Clinically apparent, microcephaly is one of many fetal  
87 brain abnormalities that can lead to later neurodevelopmental sequelae.<sup>15</sup> Although a clinical  
88 diagnosis of microcephaly with normal brain imaging does not necessarily mean impaired brain  
89 growth, a reduction in skull volume is indicative of underlying cerebral cortical volume loss.<sup>8,16</sup> A  
90 study of Finnish infants admitted to a neonatal ward for example, showed that a one standard  
91 deviation increase in head circumference at birth was associated with a 0.8-1.5 unit increase in  
92 cognitive scores at 56 months.<sup>17</sup>

93 If we use microcephaly to screen for congenital diseases, and if OFC is normally distributed in  
94 the reference population, 2.3% of the population will be classified as having microcephaly, many  
95 of whom will be healthy. Conversely, many neonates with congenital infections that affect the  
96 brain will not have severe enough manifestations to cause microcephaly. To illustrate this, we  
97 simulate two scenarios (Figure 1). A definition of <-3 z-scores (0.1% of the population)<sup>18,19</sup> is

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

105

### 106 **Pathogenesis and embryology**

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

120

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

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

132

### 133 *CMV*

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

143

### 144 *HSV*

145 HSV can infect multiple brain cell types (human<sup>35,38</sup>, in vitro<sup>42</sup> and mouse<sup>48</sup>) but how this leads

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

152

### 153 *Rubella*

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

160

### 161 *ZIKV*

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



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

190

### 191 **Infection in pregnancy: Epidemiology, transmission and clinical features**

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

194

195 *CMV*

196 Most women of childbearing age in low- and middle-income country (LMICs) have long-lasting  
197 immunity from prior CMV infection but viral reactivation can take place in pregnancy or during  
198 periods of immunosuppression. In Sub-Saharan Africa, Latin America and South Asia, over 90%  
199 of the general population have IgG antibodies compared to a seroprevalence of 40-60% in high-  
200 income countries.<sup>63-68</sup> Prevalence of congenital CMV infection in high-income countries is  
201 estimated to be 0.7% of all live births, or 1-5% in low-income countries.<sup>63,66,69</sup>

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

213

214

215 *HSV*

216 Primary microcephaly is a relatively rare complication of perinatal HSV infection and is mainly  
217 found in association with in-utero infection which accounts for only 5% of cases.<sup>75</sup> Global

218 average seroprevalence is 18% amongst women of childbearing age but this includes huge  
219 variations: from 4.1% in Japan to 62% in East Asia and 70% in Sub-Saharan Africa.<sup>76,77</sup> Neonatal  
220 infection at the time of birth from mothers with symptomatic genital infection is more common  
221 but does not cause primary microcephaly.

222

### 223 *Rubella*

224 Rubella is a vaccine-preventable disease and rates of infection are largely dependent on the  
225 coverage of immunisation programmes. The global incidence of congenital rubella syndrome has  
226 reduced from 0.1-0.2 per 1000 live births prior to vaccination to near elimination (<0.01 per  
227 100,000 live births) in areas with comprehensive vaccination rates.<sup>78</sup> Vaccination programmes  
228 were widespread in most countries in 2014 except in Sub-Saharan Africa and South Asia.<sup>79</sup>  
229 Despite widespread vaccination programmes an estimated 9.4% of pregnant women remain  
230 seronegative worldwide and therefore susceptible to infection.<sup>80</sup> In areas without vaccination  
231 programmes, incidence of congenital rubella syndrome has been estimated at 19-283 per 100,000  
232 live births in the African region and 18-309 per 100,000 live births in the South Asian region.<sup>78</sup>

233

### 234 *T. gondii*

235 Most women of childbearing age in Latin America (51-72%), Central Europe (58%), and West  
236 Africa (54-77%) have specific IgG antibodies to *T. gondii*.<sup>81</sup> Conversely, relatively few women of  
237 childbearing age are found to be seropositive in South-east Asia, China and Korea (4-39%),  
238 Scandinavia (11-28%), and the United States (15%).<sup>81,82</sup> Mother-to-child transmission  
239 predominantly occurs following primary infection during pregnancy. The global incidence of  
240 congenital toxoplasmosis is estimated at 1.5 cases per 1000 live births (95% credible interval 1.4,  
241 1.6), being highest in some Latin America countries (average, 3.4 per 1000) and lowest in parts

242 of Europe (0·5 per 1000).<sup>83</sup> The risk of congenital infection may rise due to reactivation of *T.*  
243 *gondii* from immunosuppression, for example resulting from HIV.<sup>84</sup>

244  
245 *ZIKV*  
246 *ZIKV* has been described for over 60 years with intermittent case reports until outbreaks in Yap  
247 Island (estimated 73% (95% CI 68, 77) of population infected) in 2007, French Polynesia (up to  
248 66% IgG +ve) in 2013 and Brazil in 2015.<sup>55,85,86</sup> It is currently documented in 84 countries or  
249 subregional areas and 31 have reported microcephaly or neurological malformations associated  
250 with *ZIKV*.<sup>87</sup> The prevalence of suspected disease in women of childbearing age is 5400 per  
251 100,000 in Brazil.<sup>88</sup>

252  
253 **Laboratory diagnosis during pregnancy**  
254 Any pregnant woman presenting with fever and/or rash should be evaluated for risk of infections  
255 that are potentially transmitted to the fetus. There are many infectious causes of rash, for example  
256 Parvovirus B19, measles and varicella zoster, and extensive guidelines exist describing when  
257 testing should be done.<sup>89</sup>

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

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

271

### 272 *Serological testing in pregnant women*

273 For rubella, *T. gondii* and CMV commercial enzyme immunoassays are routinely available for  
274 antibody detection. For rubella and toxoplasmosis cases, serological diagnosis of acute primary  
275 infection relies on detection of acute phase IgM antibodies, which for most do not reach adequate  
276 levels until one week following acute infection. For all three, paired IgG samples taken at least  
277 10-14 days apart should also be obtained to confirm seroconversion. A fourfold rise in antibody  
278 titre is indicative of recent although not necessarily primary infection.<sup>94</sup> *T. gondii* IgM antibodies  
279 are commonly detected by ELISA in acute infections, but may persist for several months.<sup>95</sup> High  
280 levels of IgG antibodies may also be present during the acute phase of infection.<sup>96</sup>

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

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

290  
291 *In-utero testing*  
292 In the event that acute maternal infection with any of the above pathogens is considered to pose a  
293 risk, infection of the infant can be investigated where possible by amplification of pathogen  
294 nucleic acid from amniotic fluid. Amniotic fluid samples are typically obtained at 18 weeks  
295 gestation to determine fetal infection with *T. gondii* and guide therapy.<sup>97</sup> In CMV infection, there  
296 is a 6-8 week window between maternal infection and being able to detect the virus in amniotic  
297 fluid. PCR has high sensitivity when performed at the correct time (20-21 weeks gestation or 7  
298 weeks after maternal infection) and, when combined with culture, nearly all congenital infection  
299 can be diagnosed.<sup>63</sup> PCR testing of amniotic fluid for HSV is possible but does not seem to  
300 correlate with neonatal infection,<sup>98</sup> and has recently been shown to be possible with ZIKV.<sup>99</sup>

301  
302 *Screening*  
303 Childhood immunisation programmes against rubella are routinely available in 147 countries,  
304 with an estimated global coverage of 46%.<sup>100</sup> Pregnant women may be screened for rubella  
305 antibody and those in whom levels are absent or low, offered post-partum vaccination. Recently,  
306 high coverage levels of immunisation have led to some high-income countries to drop rubella  
307 screening. Prenatal screening for toxoplasmosis is routinely undertaken in Austria, France and  
308 Slovenia and neonatal screening in Denmark, Ireland and some parts of USA and Brazil.<sup>95</sup> In  
309 France, women who develop high titres of IgM antibodies or evidence of seroconversion  
310 indicative of primary infection during pregnancy are offered fetal screening and treatment with  
311 spiramycin or pyrimethamine-sulphonamide. Severe sequelae from congenital toxoplasmosis are  
312 now rarely seen after the current approach of systematic prenatal screening and treatment was  
313 implemented in France.<sup>101</sup> Screening for CMV in some high-income countries and for ZIKV in

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

316

### 317 **Clinical presentation at birth**

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

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

336

337 Identifying Specific Infectious Causes

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

342

### 343 *CMV*

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

350

### 351 *HSV*

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

357

### 358 *Rubella*

359 Fetal abnormalities resulting from *in utero* transmission of rubella range in severity according to  
360 the gestation at the time of infection. Infection in the first trimester is associated with more severe  
361 abnormalities.<sup>106</sup> Symptomatic infection in the infant is referred to as congenital rubella syndrome



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

365

366 *T. gondii*

367 Approximately 24% of live born infants infected with *T. gondii* are symptomatic at birth.<sup>109</sup> The  
368 classic signs originally described by Sabin (chorioretinitis, microcephaly or hydrocephalus, and

369 widespread intracranial calcifications) are relatively infrequent, but highly suggestive of the

370 diagnosis of congenital toxoplasmosis. More severe manifestations occur in infections earlier in

371 gestation. Intracranial lesions, for example, are seen in up to 40% of congenital infections

372 acquired before 5 weeks of pregnancy but in less than 10% of those acquired beyond 20 weeks.<sup>109</sup>

373 12.5% of liveborn infants with congenital toxoplasmosis develop severe neurological sequelae;<sup>110</sup>

374 5% of them have microcephaly.<sup>111</sup>

375

376 *ZIKV*

377 *ZIKV* infection in pregnancy and its possible link to a range of birth defects is currently the

378 subject of intense investigation worldwide. There is now convincing evidence that *ZIKV*

379 infection in pregnancy, especially in the first trimester, is associated with an increased risk of

380 microcephaly. The risk of microcephaly associated with *ZIKV* infection was estimated as 95 per

381 10,000 infected women in first trimester in French Polynesia.<sup>112</sup> Congenital disease has

382 predominantly been seen in Brazil, but this may spread across Latin America. Brasil et al

383 followed up *ZIKV* affected pregnant women in Rio de Janeiro and found that 3.4% had

384 microcephaly and 42% had abnormal clinical or radiological findings in the first month, mostly

385 affecting the central nervous system.<sup>113</sup> The spectrum of congenital Zika syndrome (CZS)

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

397

#### 398 *Markers for Prognosis*

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

408

#### 409 **Long-term consequences and resulting disability in childhood**

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

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

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

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

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

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

455

## 456 **Conclusions**

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

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

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

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

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

493

494

495 **Panel: Clinical features suggestive of congenital infection in a neonate with**  
496 **microcephaly.**<sup>132,133</sup>

- 497 • Severe microcephaly (<-3 z-scores)
- 498 • intrauterine growth restriction
- 499 • hydrops fetalis
- 500 • seizures
- 501 • cataract and other visual abnormalities
- 502 • hearing loss
- 503 • congenital heart disease
- 504 • hepatosplenomegaly
- 505 • jaundice
- 506 • 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 <i>Herpesviridae</i> 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. <sup>134</sup>
HSV 1 & 2	Alpha herpes DNA viruses. <i>Herpesviridae</i> 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. <sup>75</sup>	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. <sup>105</sup>	aciclovir/valaciclovir given to the mother prior to delivery. <sup>135</sup>
Rubella virus	Single stranded RNA virus <i>Togaviridae</i> family	Respiratory transmission. Humans are the only known host of rubella. <sup>136</sup>	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. <sup>137</sup>	Typically a mild self-limiting illness. Maculopapular rash, lymphadenopathy, malaise, arthralgia, fever. <sup>136</sup>	Immunisation of girls to prevent individual cases and the whole population to interrupt transmission. Supportive management of the infection.
<i>T. gondii</i>	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 <i>T. gondii</i> 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



		<p>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.<sup>138</sup></p> <p>Toxoplasmosis reactivation in immunocompromised women may rarely be a cause. Blood transfusion or organ transplantation.</p> <p>The only definitive hosts are members of the cat family.<sup>138</sup></p>	weeks. <sup>109</sup>	flu-like illness are the most common clinical signs and symptoms. <sup>138</sup>	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. <sup>101</sup>
ZIKV (much knowledge still	Single stranded RNA arbovirus <i>Flaviridae</i>	The main vector is the <i>Aedes</i> mosquito. <i>A. aegypti</i> is considered of greatest global importance. <sup>139</sup>	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. <sup>140</sup>  Non-human hosts are thought mainly to include non-human primates, although evidence of infection has also been demonstrated in other mammals. <sup>139</sup>		cases. <sup>141</sup>  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. <sup>142</sup>	
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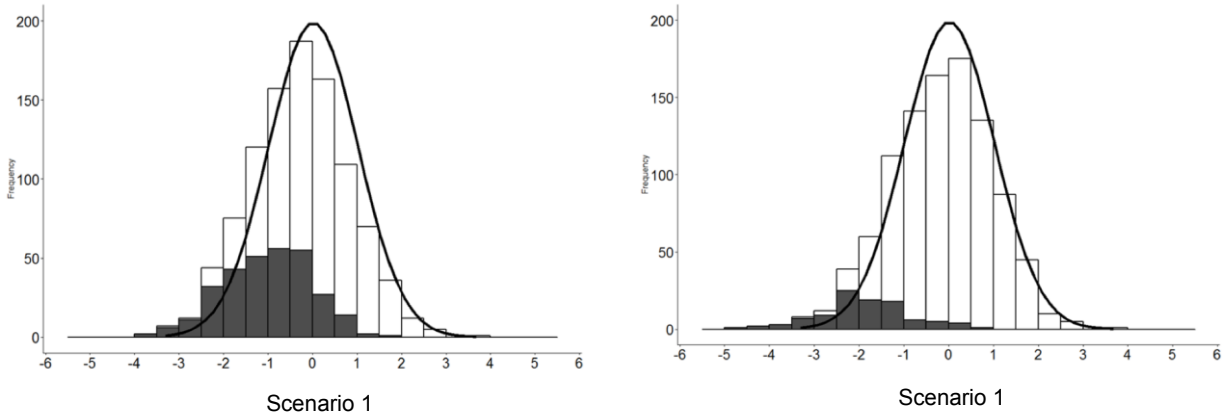
**Table 2: Diagnosis and treatment of infants**

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. <sup>143-145</sup> 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. <sup>146</sup>
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. <sup>147</sup>	Management is mainly supportive and involves monitoring for late emerging symptoms (e.g. hearing loss, endocrine problems).
<i>T. gondii</i>	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. <sup>95</sup>	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. <sup>148</sup>

ZIKV	<p>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.</p>	<p>Clinical management of complications, including dysphagia, irritability and epilepsy. Supportive care for neurocognitive delays, hearing and visual loss, and appropriate developmental follow-up.</p>
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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.<sup>149</sup>

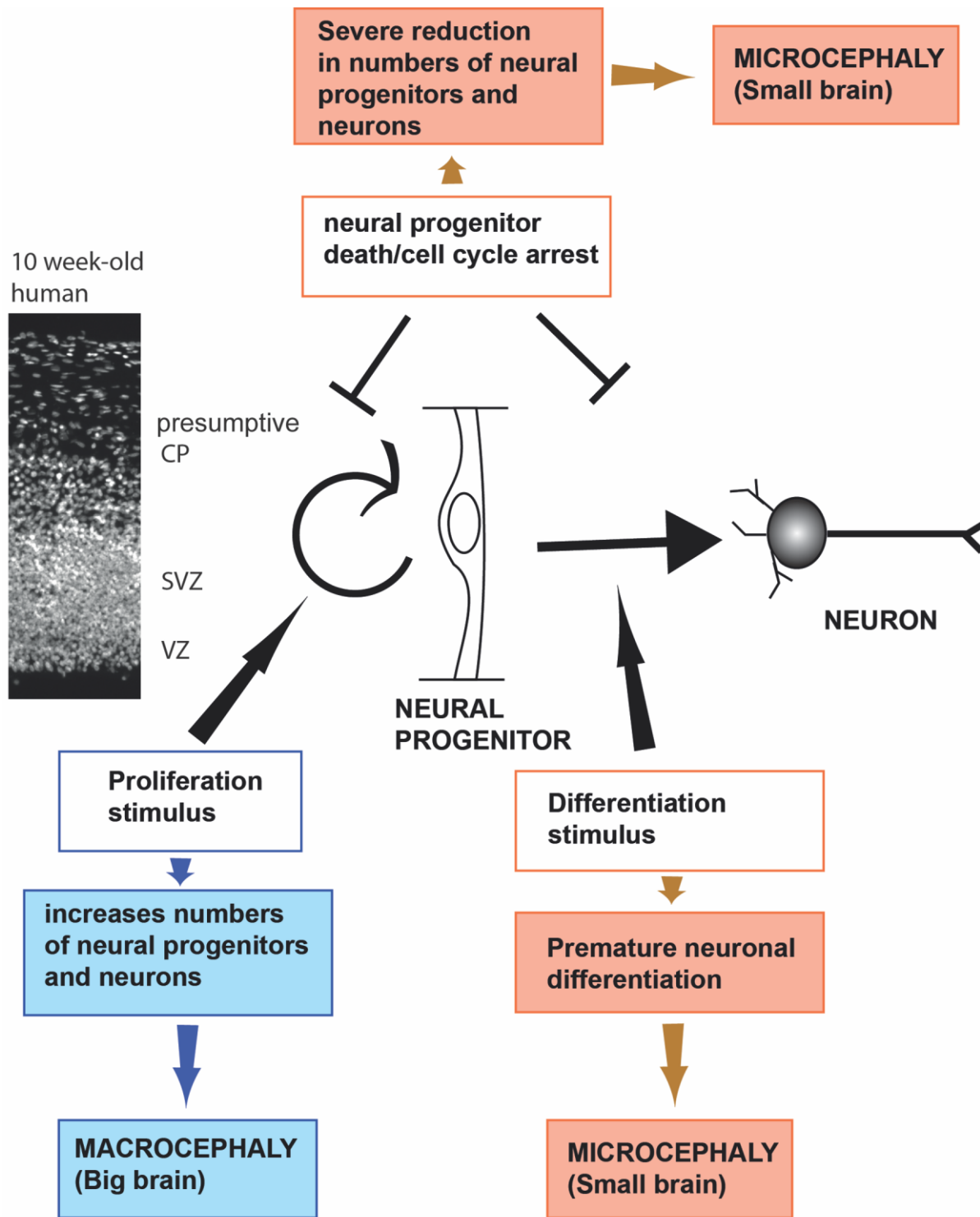
If an otherwise normal population of 1000 infants (in which children's occipito-frontal circumferences (OFCs) are close to their expected values) is exposed to a congenital infection that limits brain development, a proportion of children are labelled as “normal” by being above the  $-2$   $z$ -scores (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

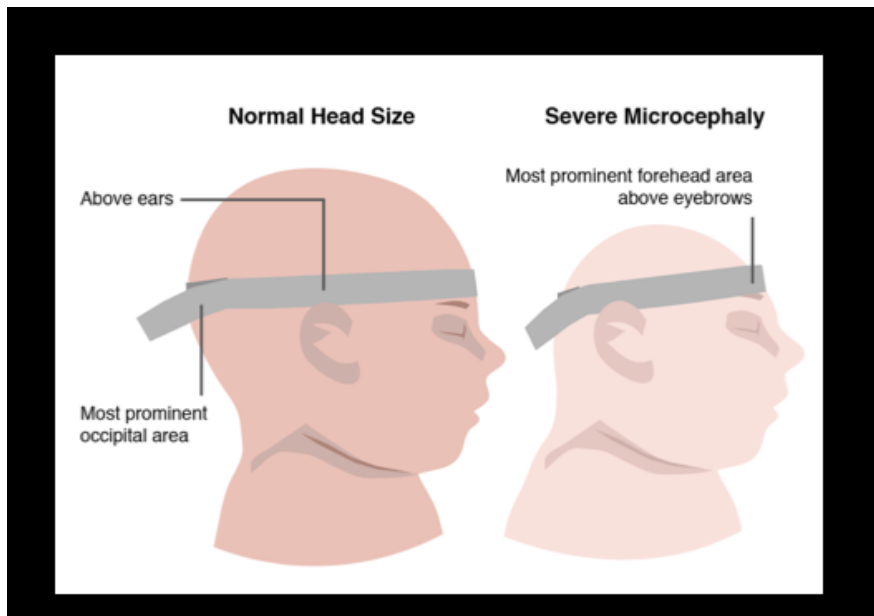
healthy population), would actually be diagnosed as “microcephalic” after the 1 SD shift caused by the infection.

**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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