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Congenital Zika syndrome: Growth, clinical, and motor development outcomes up to 36 months of age and differences according to microcephaly at birth

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ABSTRACT

Background: Little is known regarding the developmental consequences of congenital Zika syndrome (CZS) without microcephaly at birth. Most previously published clinical series were descriptive and they had small sample sizes.
Study design: We conducted a cohort study to compare the growth, clinical, and motor development outcomes for 110 children with CZS born with and without microcephaly up to their third birthday. Ninety-three had their head circumference (HC) at birth abstracted and they did not have hypertensive hydrocephalus at birth, where 61 were born with microcephaly and 32 without.
Results: The HC z-scores decreased steeply from birth to six months of age, i.e., from –3.77 to –6.39 among those with microcephaly at birth and from –1.03 to –3.84 among those without. Thus, at 6 months of age, the mean HC z-scores for children born without microcephaly were nearly the same as those for children born with microcephaly. Children born without microcephaly were less likely to have brain damage, ophthalmic abnormalities, and drug-resistant epilepsy, but the differences in many conditions were not statistically significant.
Conclusions: Children born without microcephaly were only slightly less likely to present severe neurologic impairment and to develop postnatal-onset microcephaly, and some of the original differences between the groups tended to dissipate with age.

Introduction

Congenital Zika syndrome (CZS) is characterized by congenital anomalies resulting from maternal Zika virus (ZIKV) infection during pregnancy (del Campo et al., 2017; França et al., 2016; Meneses et al., 2017; Moore et al., 2017; Schuler-Faccini et al., 2016). Microcephaly was identified as the earliest clinical sign (Schuler-Faccini et al., 2016), but neurologic and ophthalmic

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damage associated with CZS can even occur in children without microcephaly at birth (Aragao et al., 2017; Cardoso et al., 2019; van der Linden et al., 2016; da Silva et al., 2016; Walker et al., 2019). Most children born without microcephaly seek medical attention because they develop postnatal-onset microcephaly, as well as presenting with neuromotor delay or seizures in the first months of life (van der Linden et al., 2016).

CZS is recognized based on severe microcephaly with a partially collapsed skull, reduced cerebral parenchyma with subcortical calcifications, macular scarring and focal pigmentary retinal mottling, congenital contractures, and hypertonia with extrapyramidal symptoms (Moore et al., 2017). However, not all of these signs are present in every case and the full spectrum of clinical outcomes in children with CZS is yet not well understood (Carvalho et al., 2019; Gordon-Lipkin and Peacock, 2019; Lebov et al., 2019; Moore et al., 2017; Walker et al., 2019).

Little is known regarding the differences in timing of maternal ZIKV infection during pregnancy, clinical features (neuroimaging, ophthalmic damage, phenotype, and neurologic abnormalities), somatic growth, malnutrition, the head circumference (HC) trajectory, and the developmental consequences of CZS without microcephaly at birth (Gordon-Lipkin and Peacock, 2019). Most previously published clinical series were descriptive and they had small sample sizes (Aragao et al., 2017; Cardoso et al., 2019; van der Linden et al., 2016).

To address these gaps in the knowledge, we conducted a cohort study of 110 children with CZS and followed up to their third birthday.

Methods

This prospective cohort study considered children with CZS born in maternity hospitals in Maranhão State, Brazil, from March 2015 to September 2018, and they were followed for up to 36 months at the Reference Center for Neurodevelopment, Assistance, and Rehabilitation of Children (NINAR is the Portuguese acronym).

We used a slightly modified case definition of CZS based on that given by França et al. (França et al., 2016). Cases were classified into four categories and all presented physical or imaging findings: (a) confirmed by plaque reduction neutralization test (PRNT 90) > 1:10, which was performed based on a previously published protocol (Baer and Kehn-Hall, 2014), where 94 cases underwent PRNT and 40 were positive; (b) probable laboratory case with positive immunoglobulin-M enzyme-linked immunosorbent assay when PRNT was negative or not performed, with four cases; (c) highly probable clinical case with brain computed tomography (CT) injuries suggestive of CZS and negative results for STORCH (syphilis, toxoplasmosis, rubella, cytomegalovirus, and herpes simplex), with 33 cases; and (d) moderately probable clinical case with brain CT damage suggestive of CZS and incomplete or inconclusive results for STORCH, with 33 cases. qRT-PCR (quantitative real-time Polymerase Chain Reaction) for ZIKV was performed for few cases (13) and all were negative.

Brain CT was performed for children up to two years old and the images were analyzed by two radiologists with experience in neuroimaging who were blinded to the laboratory findings. The following abnormalities were considered suggestive of CZS: calcifications, reduction in cerebral parenchyma volume, secondary ventriculomegaly, malformation of cortical development, malformation/hypoplasia of the cerebellum, malformation/hypoplasia of the brain stem, and agenesis/dysgenesis of the corpus callosum (Aragao et al., 2016; Hazin et al., 2016).

Birth data were obtained retrospectively from the medical notes and the child's health card. Follow-up data were obtained at the time of consultations. Clinical data were collected by a senior neuropediatrician. We abstracted data on spasticity, epileptic seizures, age at first seizure (in months), drug-resistant epilepsy, difficulty sleeping, irritability, dysphagia, and continuous crying. We considered drug-resistant epilepsy when two or more anticonvulsant medication protocols used alone or in combination failed to control seizures in a sustained manner (Kwan et al., 2009). Maternal symptoms of ZIKV virus infection during pregnancy were considered when the mother reported rash or two of the following symptoms: fever, itching, arthralgia/joint swelling, conjunctivitis, and headache. The gestational trimester of maternal infection was categorized as first, second, or third trimester (n = 83).

Weight, length, and HC at birth were abstracted from medical notes. Weight, length, and HC at 6, 12, 24, and 36 months were collected by trained personnel following a standardized protocol (de Onis et al., 2004). HC was measured with a nonextensible tape, which was passed around the head and anchored above the eyebrows and over the occiput. Low birth weight was defined as birth weight <2500 g. Preterm birth was defined as <37 completed weeks of gestational age. Data on birth weight were unavailable for three cases and on preterm birth for five cases. Fronto-temporal retraction, craniofacial disproportion, prominent occiput, biparietal depression, and excess nuchal skin were recorded. Arthrogryposis and clubfoot were considered signs of the fetal akinesia deformation sequence.

A trained physiotherapist evaluated the degree of motor impairment for children according to the gross motor function classification system (GMFCS) (Palisano et al., 1997) validated for Brazilian-Portuguese (Hiratuka et al., 2010) into five levels ranging from level I where the child walks without limitations to level V where the child is transported in a manual wheelchair.

A senior ophthalmologist performed ocular examination by biomicroscopy and indirect ophthalmoscopy. The findings were documented with wide-angle fundus photography by using the RetCam Shuttle system (Clarity Medical Systems, Pleasanton, CA, USA). The following ophthalmic damages were examined: chorioretinal scarring, retinal pigment mobilization, optic nerve pallor, optic nerve hypoplasia, bilateral retinal alteration, and bilateral optic nerve alteration.

We used the online program INTERGROWTH-21st (Villar et al., 2014) to calculate z-scores for weight, length, and HC according to sex and gestational age at birth. Z-scores more than two standard deviations (2 SD) below the mean for gestational age and sex defined low weight for age, low length for age, and microcephaly at birth. Severe microcephaly was defined as HC z-score >3SD below the mean for gestational age and sex. Microcephaly was considered proportionate when the child also had a low weight for age or low length for age at birth, and disproportionate when the birth weight and length for age were normal (Silva et al., 2018).

Postnatal-onset microcephaly was defined as HC z-score >2 SD below the mean for sex and age. Corrected gestational age was used for preterm children. We used the World Health Organization criteria for classifying weight for age, weight for length, length for age, and HC for age (microcephaly) after birth. These parameters were considered abnormal when >2 SD below the mean for sex and age (WHO Multicentre Growth Reference Study Group, 2007, 2006).

Categorical data were described as absolute and relative frequencies. We evaluated the normality of the numerical variables based on their asymmetry, kurtosis, and boxplots. The chi-square test and Fisher's exact test were used to verify associations between microcephaly at birth and other categorical variables. Z-scores were expressed as means and 95% confidence intervals. We used the independent samples *t*-test to compare mean z-scores between the groups with and without microcephaly at birth.

Results

We obtained data on 110 cases of CSZ based on the criteria specified in the methods. In total, 95 cases had their HC at birth

abstracted and two of these had hypertensive hydrocephalus at birth so they were excluded, thereby resulting in 93 cases eligible for analyzing microcephaly at birth. Sixty-one cases (66%) presented with microcephaly at birth whereas 32 (34%) were born without microcephaly. Forty-four (47.3%) of the 93 children were born with severe microcephaly. Among the 32 cases born without microcephaly, 28 (87.5%) developed postnatal-onset microcephaly detected at any follow-up visit, and most (23, 65.6%) presented with severe postnatal-onset microcephaly.

Among the four cases that did not develop microcephaly postnatally, two had positive PRNT results with cortical/subcortical brain calcifications and two were considered highly probable clinical cases. Although they did not develop postnatal-onset microcephaly, two of the four cases exhibited reductions in HC over time.

The case fatality rate was 5.5% (n = 6) up to 36 months of age and the main causes of death were severity of the congenital anomalies due to CZS and pneumonia. Three of the six children had positive PRNT results and three were moderately probable clinical cases. One case had hypertensive hydrocephalus at birth, one was born without microcephaly, and one had severe microcephaly, and the other three had no record of the HC at birth.

Seventy-five percent of the mothers recalled the timing of the symptoms of maternal ZIKV infection during pregnancy. Infection occurred in the first trimester for two-thirds of these mothers (Table 1). Twenty-two percent of all children had low birth weights and 13.3% were born preterm. Most infants exhibited at least one morphological characteristic associated with CSZ. The most frequent characteristics were craniofacial disproportion (90.6%), frontotemporal retraction (71.7%), prominent occiput (55.7%), and biparietal depression (46.2%). Excess nuchal skin was present in 24.5%. Characteristics associated with the fetal akinesia syndrome were less common, where 9.9% had arthrogryposis and 7.9% presented with clubfoot (Table 1).

Maternal ZIKV infection was most frequently reported in the first trimester of pregnancy among those born with microcephaly (79.2%) compared with those born without microcephaly (46.1%). Prominent occiput ($p < 0.001$), biparietal depression ($p = 0.017$), and excess nuchal skin ($p = 0.012$) were also more frequently observed among those born with microcephaly than those born without (Table 1).

All 108 cases that underwent brain CT imaging presented with an abnormality. The most prevalent abnormalities were brain calcifications (93.5%), ventriculomegaly (88.8%), reduced cerebral parenchyma (85.8%), and malformations of cortical development (78.3%). Cerebellar or brain stem hypoplasia/malformation and agenesis or dysgenesis of the corpus callosum were identified in fewer than 20% of cases. Ventriculomegaly ($p = 0.002$), reduced cerebral parenchyma ($p < 0.001$), malformations of cortical development ($p = 0.047$), and brain stem hypoplasia or

Table 1
Demographic, clinical, and fetal brain disruption sequence phenotype characteristics according to microcephaly at birth among children with congenital Zika syndrome. São Luís, 2016–2018.

Characteristics	Total[a]		Microcephaly at birth (n = 93)				*p*-Value
			Yes		No		
	No.	(%)	No.	(%)	No.	(%)	
Sex (n = 110)							0.853[c]
M	65	59.1	35	57.4	19	59.4	
F	45	40.9	26	42.6	13	40.6	
Trimester of maternal infection during pregnancy (n = 83)[b]							0.010[d]
First	55	66.3	38	79.2	12	46.1	
Second	19	22.9	8	16.7	10	38.5	
Third	9	10.8	2	4.2	4	15.4	
Low birth weight (n = 107)							0.187[c]
No	84	78.5	44	72.1	27	84.4	
Yes	23	21.5	17	27.9	5	15.6	
Preterm birth (n = 105)							0.223[c]
No	91	86.7	55	90.2	26	81.2	
Yes	14	13.3	6	9.8	6	18.8	
Frontotemporal retraction (n = 106)							0.087[c]
No	30	28.3	12	20.0	11	36.7	
Yes	76	71.7	48	80.0	19	63.3	
Craniofacial disproportion (n = 106)							0.093[d]
No	10	9.4	2	3.3	4	13.3	
Yes	96	90.6	58	96.7	26	86.7	
Prominent occiput (n = 106)							<0.001[c]
No	47	44.3	16	26.7	21	70.0	
Yes	59	55.7	44	73.3	9	30.0	
Biparietal depression (n = 106)							0.017[c]
No	57	53.8	26	43.3	21	70.0	
Yes	49	46.2	34	56.7	9	30.0	
Excess nuchal skin (n = 106)							0.012[d]
No	80	75.5	39	65.0	27	90.0	
Yes	26	24.5	21	35.0	3	10.0	
Arthrogryposis (n = 101)							0.488[d]
No	91	90.1	50	86.2	26	92.9	
Yes	10	9.9	8	13.8	2	7.1	
Clubfoot (n = 101)							0.569[d]
No	93	92.1	52	89.7	28	93.3	
Yes	8	7.9	6	10.3	2	6.7	

[a] 110 children with congenital Zika syndrome were included, where 93 had their head circumference abstracted and lacked hypertensive hydrocephalus at birth.
[b] 24 asymptomatic mothers and three others who did not know if they had symptoms attributable to ZIKV infection during gestation were excluded.
[c] Chi-square test.
[d] Fisher's exact test.

Table 2
Brain computed tomography (CT) findings according to microcephaly at birth among children with congenital Zika syndrome (n = 108). São Luís, 2016–2018.

Brain CT findings	Total[a]		Microcephaly at birth (n = 93)				*p*-Value
			Yes		No		
	No.	(%)	No.	(%)	No.	(%)	
Brain calcification (n = 108)							0.338[c]
No	7	6.5	2	3.3	3	9.4	
Yes	101	93.5	58	96.7	29	90.6	
Main site of calcifications (n = 100)							0.114[c]
Cortical/subcortical	69	69.0	40	69.0	21	72.4	
Periventricular	3	3.0	0	0	2	6.9	
Thalamus and basal ganglia/diffuse	28	28.0	18	31.0	6	20.7	
Ventriculomegaly (n = 107)							0.002[c]
No	12	11.2	1	1.7	7	21.9	
Yes	95	88.8	59	98.3	25	78.1	
Reduced cerebral parenchyma (n = 106)							<0.001[b]
No	15	14.2	2	3.4	10	31.3	
Yes	91	85.8	57	96.6	22	68.7	
Malformations of cortical development (n = 106)							0.047[b]
No	23	21.7	7	11.7	9	28.1	
Yes	83	78.3	53	88.3	23	71.9	
Cerebellar hypoplasia or malformation (n = 105)							0.273[c]
No	85	81.0	45	76.3	28	87.5	
Yes	20	19.0	14	23.7	4	12.5	
Brainstem hypoplasia or malformation (n = 107)							0.045[c]
No	86	80.4	45	76.3	30	93.7	
Yes	21	19.6	14	23.7	2	6.3	
Agenesis or dysgenesis of the corpus callosum (n = 107)							0.340[c]
No	99	92.5	57	96.6	29	90.6	
Yes	8	7.5	2	3.4	3	9.4	

[a] 110 children with congenital Zika syndrome were included, where 93 had their head circumference abstracted and lacked hypertensive hydrocephalus at birth.
[b] Chi-square test.
[c] Fisher's exact test.

malformation ($p = 0.045$) were most frequently identified among those born with microcephaly (Table 2).

The characteristics of faciobrachial dystonic seizure (FBDS) were observed in a child who was born with severe microcephaly and presented with moderate brain volume loss, multiple parenchymal calcifications, and malformation of cortical development (Figure 1). A milder phenotype was seen in another child born without microcephaly who had slight brain volume loss, sparse parenchymal calcifications, and small occipital bone prominence (Figure 2).

Among the neurologic abnormalities, spasticity (97%) and epileptic seizures (90.7%) were the most frequent. Difficulty sleeping, irritability, continuous crying, and dysphagia were present in most cases. In 58.8% of cases, the age at first epileptic seizure was <6 months and 60% had drug-resistant epilepsy. Ninety percent of all cases were at GMFCS level V. Drug-resistant epilepsy ($p = 0.016$), spasticity ($p = 0.032$), and continuous crying ($p = 0.014$) were most common among those who presented with microcephaly at birth. Furthermore, the severity of motor disability was significantly higher in those with microcephaly at birth ($p < 0.001$) (Table 3).

Ophthalmic damage was observed in 43.9% of cases and the most frequent findings were in the retina with chorioretinal scar (22.5%) and focal pigmentary mottling (24.5%). Optic nerve abnormalities were also found, where atrophy was detected in 19.4% and hypoplasia in 5.1%. Most damage to the retina and optic nerve was bilateral. Damage to the anterior segment of the eye was observed less frequently (5.1%). Presence of any ophthalmic damage ($p = 0.013$) and bilateral retina damage ($p = 0.045$) were also more frequently diagnosed among those with microcephaly at birth (Table 4). There were no statistically significant differences in the presence and absence of microcephaly at birth for the other conditions listed in Tables 1–4 ($p \geq 0.05$).

The mean weight for age, weight for length, and length for age z-scores tended to decrease slightly from birth to 36 months (Figure 3). However, the mean HC z-scores decreased steeply from –2.83 at birth to –5.71 at 6 months, before remaining nearly stable up to 36 months of age (Supplementary Table 5).

The mean weight for age z-scores were lower at birth and at 12 and 24 months of age for those born with microcephaly (Figure 3A), whereas the mean weight for length differed between the two groups only at birth. Children without microcephaly had a normal mean weight for length whereas those with microcephaly did not, but the curves converged to a value of –1 for both groups over time (Figure 3B). The mean length for age z-scores differed significantly between the two groups at six, 12, and 24 months of age, and consistent decreases in the z-scores were observed in both groups over time (Figure 3C). The mean HC for age z-scores differed at all ages between the two groups. The HC for age z-scores decreased steeply in both groups from –3.77 at birth to –6.39 at 6 months of age among those born with microcephaly, and from –1.03 at birth to –3.84 at 6 months of age among those born without microcephaly. Thus, at 6 months of age, the mean HC for age z-scores of children born without microcephaly were nearly the same as those of children born with microcephaly at birth (Supplementary Table 5) (Figure 3D).

Thirteen percent of cases had a low weight for age and 38.7% had a low length for age at birth (Supplementary Table 6). Among those born with microcephaly, 82% did not have a low gestational age at birth, but they were disproportionate due to their microcephaly. By contrast, 51.9% were disproportionate according to their length at birth, i.e., the length for age at birth was normal but microcephaly was present. Among those born without microcephaly, most were proportionate but 6.2% had a low weight for age and 18.5% had a low length for gestational age at birth. Malnutrition measured by the weight for age z-score increased

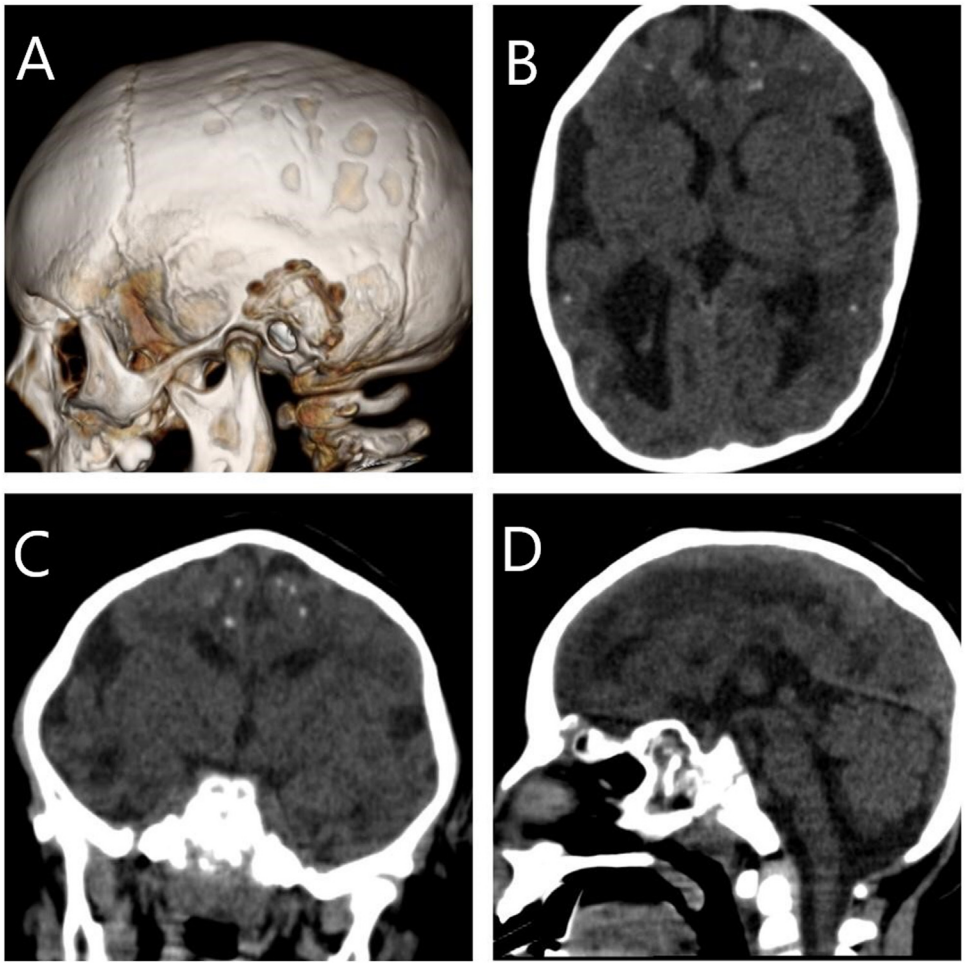

Figure 1. Computed tomography with lateral view of 3D skull reconstruction (A) and multiplanar reformatting (B, C and D – axial, coronal and sagittal, respectively) of the brain parenchyma, showing moderate brain volume loss, multiple parenchymal calcifications and malformation of the cortical development. São Luís, 2016-2018.

from 12.9% at birth to 51.6% at 24 months, before remaining stable thereafter. According to the low weight for length values, malnutrition increased from 11.3% at birth to 46.3% at 36 months. At some ages, malnutrition tended to be higher among those born with microcephaly compared with those born without microcephaly (Supplementary Table 6).

The fraction of children with low weight for age increased in both groups over time up to 24 months before then stabilizing, with a higher fraction observed in children born with microcephaly (Figure 4A). The two groups had similar fractions of children with a low weight for length and these fractions both increased over time (Figure 4B). Low length for age remained constant over time but it was higher in the group with microcephaly (Figure 4C). The low HC for age fraction of children born without microcephaly increased greatly during the first 6 months of age (Figure 4D).

Discussion

Most cases of CZS were severe and they exhibited a typical FBDS phenotype (Moore et al., 1990), but some also presented less frequently with a fetal akinesia deformation sequence phenotype (Melo et al., 2016). Almost all (90.7%) presented with convulsions, which appeared more often before the age of six months (Carvalho et al., 2020), and they had severe motor impairment in infancy and early childhood (A. Ventura et al., 2020; Melo et al., 2019). Consequently, most patients were unable to walk or sit without help at an average age of 25.5 years. Ophthalmic damage occurred in 43.5% and 60% had drug-resistant epilepsy. From birth to six months of age, a sharp reduction was observed in the HC for age z-scores from 3SD to 6SD below the mean, before stabilizing subsequently. Most children exhibited a disproportionate fetal growth pattern where brain growth was disrupted but the femur grew normally (51.9%), with the so-called femur sparring intrauterine growth retardation phenotype (Walker et al., 2019). Approximately half were malnourished at 36 months of age.

It is recognized that microcephaly is one of the components of CZS and that neurologic and ophthalmic damage can occur without microcephaly (van der Linden et al., 2016; Ventura et al., 2016), and it may even be asymptomatic (Oliveira et al., 2020). In our case series, slightly more than one-third (34.4%) were born without microcephaly but nearly all of the cases (87.5%) developed postnatal-onset microcephaly during infancy.

We compared the clinical features, growth, and development among children born with and without microcephaly, where most clinical features did not differ between the two groups. However, children who did not have microcephaly at birth were less likely to be infected in the first trimester of pregnancy. They also tended to have less severe brain CT abnormalities and a lower prevalence of ophthalmic injuries. Furthermore, some of them developed with less severe motor function impairments and they typically presented with normal growth during pregnancy. However, from birth to 6 months of age, their HC for age z-scores decreased sharply from 1SD to 4SD below the mean. The clinical presentation of those born without microcephaly appeared to be less severe

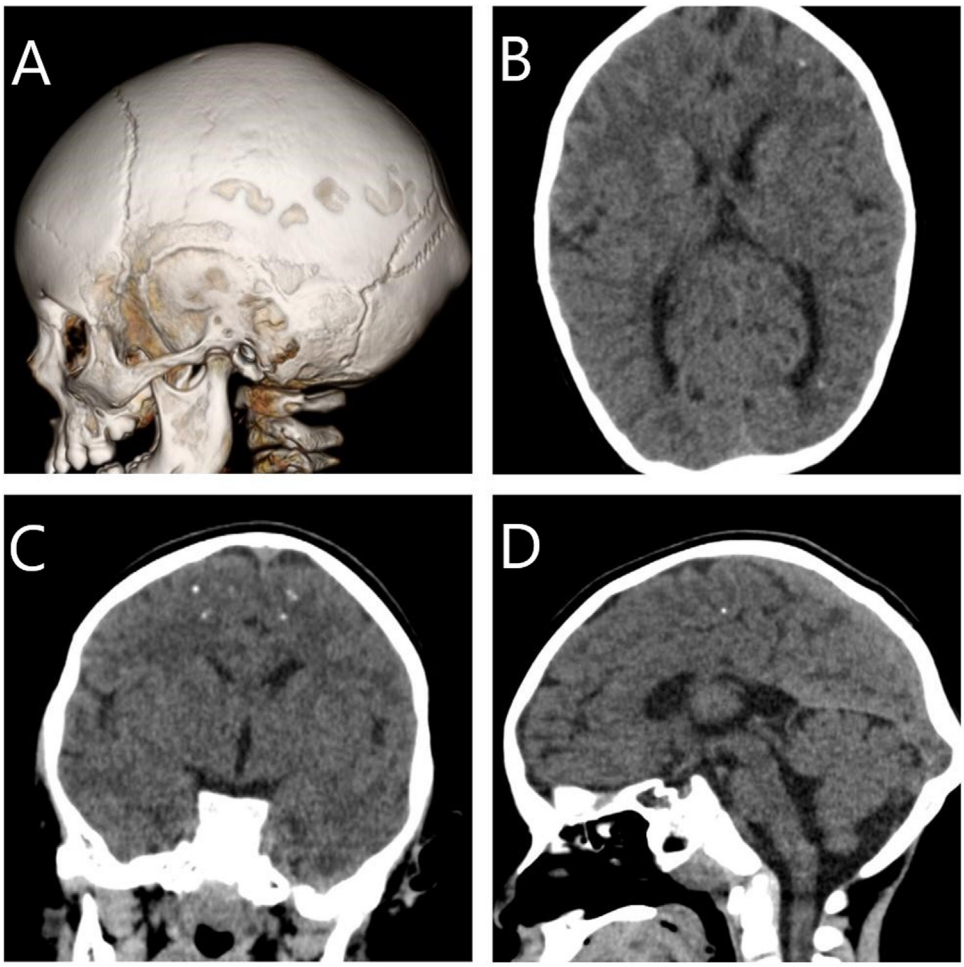

Figure 2. Computed tomography with lateral view of 3D skull reconstruction (A) and multiplanar reformatting (B, C and D – axial, coronal and sagittal, respectively) of the brain parenchyma, showing slight brain volume loss, sparse parenchymal calcifications and small occipital bone proeminence. São Luís, 2016-2018.

compared with those born with microcephaly, but at around 2 years of age, only 10% of those children born without microcephaly were able to walk without the need for any assistive mobility device and be expected to walk without limitations (GMFCS level I), whereas 6.4% were able to pull to stand on a stable surface and cruise short distances, with a prognosis of walking using a hand-held mobility device (GMFCS level III) at around 2 years of age.

One of the strengths of our study is that the sample size allowed us to detect some differences between the groups born with and without microcephaly. Furthermore, our study is one of the largest clinical series published to date with a longer follow-up duration. However, it also had some limitations. We only included patients who attended a referral center, so it was biased toward the most severe cases. In addition, it was prone to recall bias regarding the timing of ZIKV infection during pregnancy. The attrition rate was high at 36 months (60.9% = 67 out of 110) but we were able to follow 86.4% of cases (95 out of 110) until 24 months. Among the 110 cases, 39% had laboratory confirmation of ZIKV infection. PRNT was performed after 18 months of age, and thus passive transfer of maternal antibodies was highly unlikely to still be present. A positive PRNT can also indicate postnatal exposure to ZIKV but this is unlikely because all cases presented with brain damage or were IgM positive when they had their first consultation with a neuropediatrician in the first months of life. Cross-reactivity with other flaviviruses, especially dengue, is also unlikely because dengue does not produce these clinical outcomes.

In contrast to our expectations, 54 cases (57%) had a negative PRNT result despite presenting with clinical signs and symptoms compatible with CZS, and three of the four cases who had Zika-related IgM had negative PRNT results, thereby strongly suggesting a high false negative rate among the PRNT results. In agreement with our findings, a previous study reported that among 99 mothers who were qRT-PCR positive for ZIKV, only 48.5% were PRNT positive (Ximenes et al., 2019). Therefore, no currently available test can confirm ZIKV infection other than qRT-PCR. Given the high false negative rates with PRNT, CZS mainly requires a clinical diagnosis (Gordon-Lipkin and Peacock, 2019).

Most of the presentations assessed did not differ according to microcephaly at birth. Overall, only clinical, neurologic, brain CT, ophthalmic, and motor function findings related to severe brain damage were worse among those born with microcephaly. The frequency of epileptic seizures did not differ between those with or without microcephaly at birth, but drug-resistant epilepsy was most frequent among infants born with microcephaly. Infants without microcephaly at birth presented with normal HC for age z-scores and were born without intrauterine growth restriction but their HC for age z-scores decreased greatly at 6 months of age, where they reached values close to those of infants with microcephaly at birth and nearly all developed microcephaly postnatally. By contrast, the HC for age z-scores of infants with microcephaly also decreased greatly from birth to 6 months of age but they stabilized subsequently. These great declines in the HC

Table 3
Clinical features and gross motor function according to microcephaly at birth among children with congenital Zika syndrome. São Luís, 2016–2018.

Clinical features	Total[a]		Microcephaly at birth (n = 93)				*p*-Value
			Yes		No		
	No.	(%)	No.	(%)	No.	(%)	
Spasticity (n = 101)							0.032[d]
No	3	3.0	0	0.0	3	10.7	
Yes	98	97.0	58	100	25	89.3	
Epileptic seizures (n = 108)							0.220[d]
No	10	9.3	3	4.9	4	12.9	
Yes	98	90.7	58	95.1	27	87.1	
Age at first epileptic seizures (n = 97)							0.956[c]
<6 months	57	58.8	34	58.6	16	59.3	
≥6 months	40	41.2	24	41.4	11	40.7	
Drug-resistant epilepsy (108)							0.016[c]
No	43	40.0	17	27.9	17	53.1	
Yes	65	60.0	44	72.1	15	46.9	
Difficulty sleeping (n = 101)							0.918[c]
No	25	24.8	13	22.4	6	21.4	
Yes	76	75.2	45	77.6	22	78.6	
Irritability (n = 101)							0.281[c]
No	35	34.7	18	31.0	12	42.9	
Yes	66	65.3	40	69.0	16	57.1	
Dysphagia (n = 101)							0.064[c]
No	42	41.6	19	32.8	15	53.6	
Yes	59	58.4	39	67.2	13	46.4	
Continuous crying (n = 101)							0.014[c]
No	41	40.6	19	32.8	17	60.7	
Yes	60	59.4	39	67.2	11	39.3	
Gross motor function classification system (GMFCS)[b] (n = 102)							<0.001[d]
I	3	2.9	0	0.0	3	9.7	
II	0	0	0	0.0	0	0.0	
III	2	2.0	0	0.0	2	6.4	
IV	5	4.9	0	0.0	4	12.9	
V	92	90.2	56	100.0	22	71.0	

[a] 110 children with congenital Zika syndrome were included, where 93 had their head circumference abstracted and lacked hypertensive hydrocephalus at birth.
[b] GMFCS: Level I, walks without limitations; II, walks with limitations; III, walks using a hand-held mobility device; IV, self-mobility with limitations and may use powered mobility; V, transported in a manual wheelchair.
[c] Chi-square test.
[d] Fisher's exact test.

Table 4
Ophthalmic injuries according to microcephaly at birth among children with congenital Zika syndrome. São Luís, 2016–2018.

Ophthalmic injuries	Total[a]		Microcephaly at birth (n = 93)				*p*-Value
			Yes		No		
	No.	(%)	No.	(%)	No.	(%)	
Any ophthalmic damage (n = 98)							0.013[b]
No	55	56.1	28	49.1	21	77.8	
Yes	43	43.9	29	50.9	6	22.2	
Damage to the anterior segment of the eye (n = 98)							0.241[c]
No	93	94.9	56	98.2	25	92.6	
Yes	5	5.1	1	1.8	2	7.4	
Chorioretinal scar (n = 98)							0.098[c]
No	76	77.5	40	70.2	24	88.9	
Yes	22	22.5	17	29.8	3	11.1	
Focal pigmentary retinal mottling (n = 98)							0.100[c]
No	74	75.5	41	71.9	24	88.9	
Yes	24	24.5	16	28.1	3	11.1	
Optic nerve atrophy (n = 98)							0.567[c]
No	79	80.6	45	78.9	23	85.2	
Yes	19	19.4	12	21.1	4	14.8	
Optic nerve hypoplasia (n = 98)							0.542[c]
No	93	94.9	56	98.2	26	96.3	
Yes	5	5.1	1	1.8	1	3.7	
Bilateral retina damage (n = 98)							0.045[c]
No	77	78.6	41	71.9	25	92.6	
Yes	21	21.4	16	28.1	2	7.4	
Bilateral optic nerve damage (n = 84)							0.208[c]
No	80	81.6	46	80.7	25	92.6	
Yes	18	18.4	11	19.3	2	7.4	

[a] 110 children with congenital Zika syndrome were included, where 93 had their head circumference abstracted and lacked hypertensive hydrocephalus at birth.
[b] Chi-square test.
[c] Fisher's exact test.

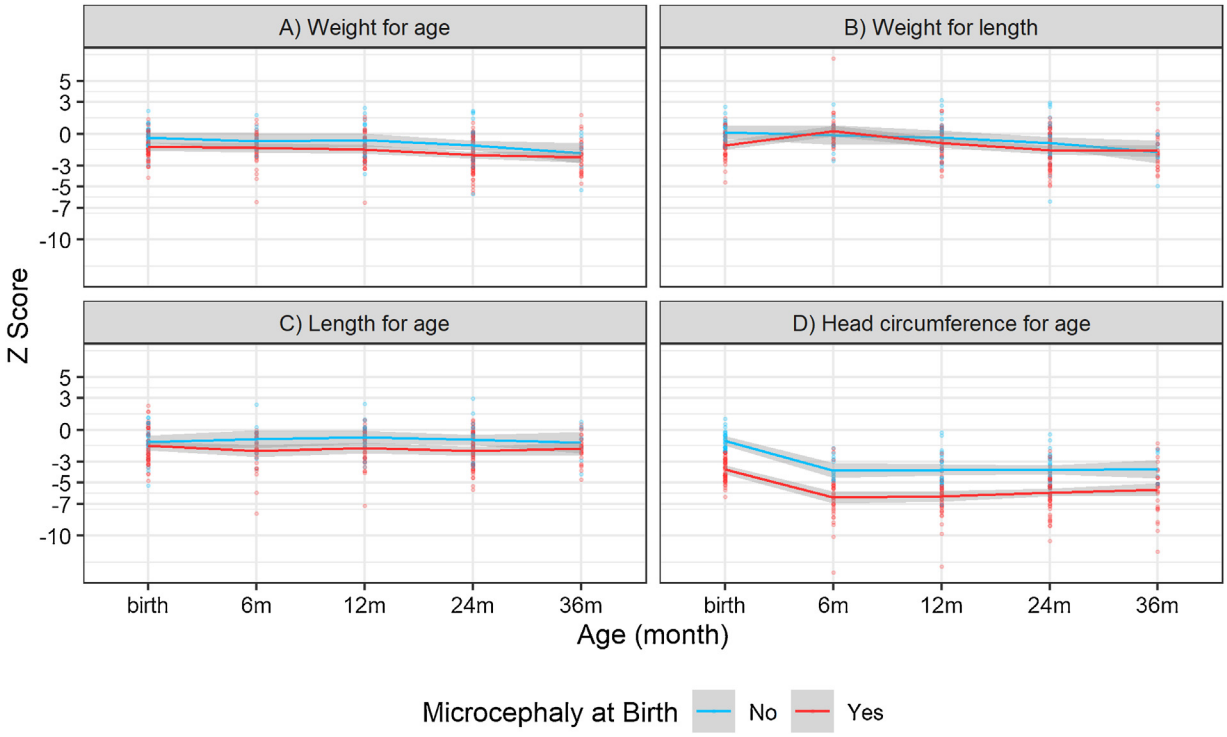

Figure 3. Z-scores at birth and 6, 12, 24, and 36 months of age for weight for age, weight for length, length for age, and head circumference for age in children with congenital Zika syndrome with microcephaly (red) or without microcephaly (blue) at birth. São Luís, 2016–2018.
Blue and red lines represent mean values, and shaded areas denote 95% confidence intervals.

Figure 4. Fraction of children (%) with congenital Zika syndrome at birth and 6, 12, 24, and 36 months of age with low weight for age, low weight for length, low length for age, and low head circumference for age with microcephaly (red) or without microcephaly (blue) at birth. São Luís, 2016–2018.

for age z-scores might suggest persistent ZIKV infection (Aid et al., 2017; Bhatnagar et al., 2017), continuing inflammation after birth (Lima et al., 2019; de Oliveira et al., 2019), or that the effect of neuroprogenitor cell destruction became more apparent over time because the brain parenchyma that develops from these cells increases in unaffected infants but not in Zika-affected infants. The stabilization of the HC for age z-scores in both groups at 6 months of age, although at lower levels in infants with

microcephaly, indicates that the damage associated with ZIKV might not continue after that period. Somatic growth was also compromised (Adachi et al., 2019; Prata-Barbosa et al., 2019), where the mean weight for age, weight for length, and length for age z-scores decreased slightly from birth to 36 months and they tended to be lower among children born with microcephaly at some ages. Almost 50.0% of cases developed malnutrition at 36 months of age.

In conclusion, a severe phenotype characterized by microcephaly at birth and FBDS was present in most children. However, another milder phenotype without microcephaly at birth was also evident. CZS children born without microcephaly were equally affected by most of the conditions described as those born with microcephaly. The only main difference observed was in terms of GMFCF. Children born with microcephaly had much worse motor development than children born without microcephaly, which can probably be explained by the damage caused to the brain during crucial in utero developmental stages. Most children born without microcephaly tended to develop postnatal-onset microcephaly and some of the original differences between the groups tended to dissipate with age.

Ethical approval

This study was approved by the Research Ethics Committee of the Federal University of Maranhão for opinion number 1510315. All mothers or guardians signed a written consent form.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2021.02.072.

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