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Clinical and neurodevelopmental outcomes based on brain imaging studies in a Colombian cohort of children with probable antenatal Zika virus exposure

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Abstract

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Institutional Review Board Statement: The clinical follow-up of this cohort was developed as part of the national public health response to the ZIKV outbreak in Colombia. It was considered public health practice by INS per Colombian legislation *(República de Colombia)*, which is in compliance with the Declaration of Helsinki. At each evaluation, which was voluntary, parents or legal guardians were informed about the procedures involved during the visits and provided written consent when accepting their childś participation. The use of clinical pictures and abstraction of clinical data considered relevant to the study was authorized by parents. The consent indicates that data provided could be used for academic purposes.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors do not have any conflicts of interest.

Background: Our aim was to describe the neuroimaging and clinical evaluations of children with antenatal Zika-virus (ZIKV) exposure.

Methods: The Colombian National Institute of Health performed serial clinical evaluations of children with probable antenatal ZIKV exposure (i.e., born to ZIKV symptomatic mothers or born with birth defects compatible with ZIKV infection, regardless of laboratory results) over two years that included head circumference (HC), eye examination, and neurodevelopmental assessments. Clinical neuroimaging studies (head computed tomography and/or brain magnetic resonance imaging) were analyzed for abnormalities, two-dimensional measurements were made of the right and left frontal and occipital cortical thickness. Two abnormal patterns were defined: Pattern 1 (sum of four areas of cortex <6 cm) and Pattern 2 (sum of four areas of cortex ≥6 cm and <10 cm).

Results: Thirty-one children had a neuroimaging study; in 24, cortical thickness was measured. The median age at the first visit was 8 (range:6–9) months and 22 (range:19–42) months at the last evaluation. In the 24 cases with cortical measurements, 3 were normal, 12 were in Pattern 1, and 9 were in Pattern 2. Children within Pattern 1 had lower mean HC at birth and in follow-up (both P<.05) and a higher frequency of structural eye abnormalities (P<.01). A trend towards poorer neuromotor development was seen in Pattern 1, although not statistically significant (P=.06).

Conclusion: Brain imaging classification based on cortical measurements correlate with ophthalmologic abnormalities and HC. Cortical thickness may be a marker for clinical outcomes in children with congenital ZIKV infection.

Keywords

congenital abnormalities; neuroimaging; developmental disabilities; eye abnormalities; microcephaly

Introduction

Since its recognition as a human teratogen, much has been learned about Zika virus (ZIKV) infection during pregnancy and its relation to congenital abnormalities of the brain and eye (Zika-associated birth defects [ZBD]). Abnormal body tone, swallowing dysfunction, epilepsy, and developmental delay occur as neurologic sequelae among infants born to mothers with probable antenatal ZIKV infection (Cranston, 2020; Lima, 2019; Olson, 2019; Pereira 2020). Children with *in utero* ZIKV exposure are now reaching their fifth year of life in some regions of Latin America. Long-term follow-up of these children has helped elucidate a range of clinical manifestations and outcomes associated with antenatal ZIKV exposure (Mulkey, 2020; Nielsen-Saines, 2019).

Following the ZIKV outbreak in Colombia (October 2015 to July 2016), the Colombian National Institute of Health (Instituto Nacional de Salud, INS), in collaboration with a multidisciplinary team, implemented a comprehensive clinical follow-up program for children with probable antenatal ZIKV exposure. This program was developed in two Colombian cities with a substantial burden of ZIKV disease to provide exposed children the necessary clinical and neurologic assessments. Children with probable antenatal ZIKV exposure were followed for over two years, and clinical and neurologic outcomes were

able to be correlated with neuroimaging findings. Although a large amount of literature has described the array of clinical and neuroimaging compromise seen in children with antenatal ZIKV infection, the correlation between structural brain compromise and long-term clinical outcomes are not fully understood. The objective of this study was to describe the neuroimaging and longitudinal clinical evaluations within this cohort of children.

Methods

Study Population

As part of a clinical follow-up program developed in two Colombian cities, INS evaluated a cohort of children with probable antenatal ZIKV exposure who were selected on at least one of two criteria. Children born to mothers registered in the national ZIKV surveillance system (SIVIGILA) from October 2015 to January 2017 met the first criterion. Their mothers met the SIVIGILA ZIKV disease case definition of fever, rash and one or more of the following symptoms (not explained by other medical conditions): non-purulent conjunctivitis or conjunctival hyperemia, arthralgias, myalgia, headache, pruritus or malaise *(Instituto Nacional de Salud, 2020)*. Children reported to the national birth defects surveillance system from December 2015 to January 2017 with clinical abnormalities possibly related to antenatal ZIKV exposure met the second criterion (Cuevas Ortiz, 2016). Laboratory evidence of ZIKV infection during pregnancy for the mother or during the postnatal period for the infant was not required for eligibility in either system since laboratory data were not included as part of the case definition, due to the complexities associated with ZIKV testing (Galang, 2020). Children identified via any of these two surveillance systems were considered as having probable *in utero* ZIKV exposure, regardless of their clinical status or presence of microcephaly and were invited to attend the comprehensive clinical follow-up program. For this analysis, we include children who attended the clinical evaluations with available clinical brain imaging. Parents/caregivers were invited via telephone to participate in the clinical evaluations with their children every 4 months in year 2017 and once every year for 2018 and 2019.

Comprehensive Clinical Evaluations

Clinical data abstraction: Parents were asked to bring prenatal, infant birth, and postnatal medical records, including imaging studies performed during the pre- and postnatal periods. Medical records were reviewed, and information was abstracted in standardized data collection forms. When available, electronic duplicates of imaging studies were obtained.

Anthropometric assessment: Children's weight, length, and head circumference (HC) were measured at every evaluation. Weight was measured using an electronic scale with readings to the nearest 10 grams. Length was measured using a portable measuring board with readings to the nearest 0.1 centimeter (cm). HC measurements were taken three times using a flexible paper non-stretchable measuring tape and the highest reading was retained and recorded to the nearest 0.1 cm. Microcephaly was defined as an HC more than 2 SD below the mean for age and sex (or gestational age [GA] for birth measurements) (Ashwal, 2009).

Clinical evaluations: A multidisciplinary team including a pediatrician, a child neurologist, and a physical medicine and rehabilitation specialist performed clinical evaluations and recorded information about neurologic impairment, epilepsy, swallowing difficulties, and developmental milestones. Pediatric ophthalmologists assessed the fundus through indirect ophthalmoscopy with thirty-dimensional (30D) lens after pupil dilation.

Neuroimaging Analysis

Children had clinical brain or other body system imaging as deemed necessary by their medical providers. Digital copies of the imaging were obtained to be reviewed by a clinician with expertise in neuroimaging (SBM). All brain images were reviewed regardless of the clinical status of the child and abnormal brain findings were recorded. The imaging modality (computed tomography [CT], radiography, magnetic resonance imaging [MRI]); and type (brain, skeletal, swallow study, etc.) was recorded for all studies.

Brain MRIs were opened in RadiANT Digital Imaging Communications in Medicine (DICOM) Viewer (Medixant, Poznand, Poland) for two-dimensional (2D) measurements and were reported to the nearest 0.1 cm (Supplemental Figure). This type of measurement was used since it could be performed on routine clinical brain MRI or head CT images. The distance between the outer portion of the cortical mantle to the ventricular lining in the right and left frontal lobes (at the level of the frontal horn) and the distance between the cortical mantle to the ventricular lining on the right and left occipital lobe was measured as the cortical thickness. The width of the right and left lateral ventricles were measured at the level of the atrium on an axial image. When brain MRI and head CT were available, the brain MRI was used for measurements. The linear length of the corpus callosum was measured from the genu to the splenium on a mid-sagittal image.

Children with microcephaly were divided into two severity patterns based on cortical measurements. Infants with a sum of the right and left frontal and right and left occipital cortex 2D measurements of less than 6 cm were classified in “Pattern 1”, reflecting a more severe cortical atrophy and damage to neural progenitor cells. Children with a sum of the right and left frontal and right and left occipital cortex of 6 cm to 10 cm were classified in “Pattern 2” as having a less severe cortical atrophy. Children with normal brain imaging findings and normal head size had a sum of cortical thickness greater than 10 cm. Brain images were analyzed, and patients were assigned to the two patterns, independent of clinical follow-up data. The measurement cut points were assigned for the two patterns after measurement of all the children’s imaging. About half of the children had values below 6 cm and the others with abnormal brain imaging had values above 6 cm but less than 10 cm, providing a cut-point of 6 cm that seemed to differentiate the severity of cortical thinning among the children with microcephaly. The method of imaging measurement and definition of each pattern was reviewed with an experienced pediatric neuroradiologist.

Data analysis

Data were organized using Microsoft Excel® (version 2016) and analyzed using SAS version 9.4 (SAS Institute, Cary, North Carolina). Fisher’s exact tests were used to compare frequency distribution of categorical variables, and the Wilcoxon Rank Sum test was used

to compare continuous variables (i.e. HC Z-scores). Data were analyzed by two independent team members, assuring accuracy and replication of the results.

For anthropometric measurements taken between 0–14 postnatal days, percentiles and standard deviations for weight, length, and HC were calculated using INTERGROWTH 21st® international standards for size at birth according to GA and sex (Villar, 2014). For measurements taken at subsequent study visits, percentiles and SD were calculated using World Health Organization (WHO) standards according to sex and age, adjusting age for preterm infants until 24 months after birth (De Onis, 2015). HC trajectory for children was plotted over time by Z-score.

Ethics

The clinical follow-up of this cohort was developed as part of the national public health response to the ZIKV outbreak in Colombia. It was considered public health practice by INS per Colombian legislation (República de Colombia; República de Colombia). At each evaluation, parents or legal guardians were informed that follow-up was voluntary and did not impact any other medical services. Parents provided written consent for review of medical records and imaging. Participant anonymity was maintained throughout the visits.

Results

Cohort Characteristics

Between March 2017 and August 2019, 60 children were initially evaluated across the two participating sites. Eleven infants identified through national birth defects surveillance were excluded due to either lack of clinical evidence of maternal ZIKV infection (n=6) or presence of birth defects incompatible with congenital ZIKV infection (n=5). Of the remaining 49 children, 31 (63%) had brain imaging (CT and/or brain MRI) and clinical data and were included in this analysis.

For the 31 infants with neuroimaging, the median maternal age at delivery was 23 (range: 17–34) years (Table 1). Twenty-seven (85%) mothers had symptoms compatible with ZIKV disease during pregnancy (Table 1). Four mothers had no ZIKV-related symptoms, but in three, symptoms were reported by their male partner. Trimester of symptoms for one mother was unknown. Laboratory testing by real-time reverse-transcription-polymerase chain reaction (rRT-PCR) or IgM, in either mother or child was available for 18 (58%) children; 10 (32%) had a positive result.

Among the 31 infants, the median gestational age at birth was 38 (range: 30–41) weeks; five infants (16%) were born premature (<37 weeks) and five (17%) infants were classified as small for gestational age (SGA). Most children were male (17/31, 55%). More than half of the children (20/31) attended at least three of the five follow-up visits (data not presented). The median age at their first visit was 8 (range: 6–9) months, and the median age at their last evaluation was 22 (range: 19–42) months.

HC at birth was available for most (26/31; 84%) children, of which 46% (12/26) had microcephaly (HC < −2 SD). Out of the five children without birth HC data, at the last

evaluation three (60%) children had microcephaly and two (40%) children had a HC in the expected range for age (data not shown) (Table 1). Eleven of 14 (79%) children with a normal HC at birth, had microcephaly at follow-up (data not shown). Therefore, at the last evaluation 84% of children had microcephaly, of which 45% was postnatal.

Twenty-six of 31 children (84%) had a clinical diagnosis of ZBD, including any structural eye or brain abnormalities (Supplemental Table). Within this subgroup, only one child (ZM28) had normal head size, no brain abnormalities, and normal development, and was included as having a ZBD because of severe left eye structural and functional abnormalities considered to be related to prenatal ZIKV exposure. Out of the five children without ZBD, only one child (ZM10), had postnatal-onset microcephaly, but had history of low Apgar scores at birth, neonatal seizures, and brain imaging compatible with birth asphyxia. Of the four children without any brain or eye structural abnormalities, two (ZM17 and ZM27) had normal physical and neurologic assessments; one child (ZM05) had hypotonia, developmental delay, and autistic spectrum disorder (ASD); and one child (ZM16) was classified as having hypotonia, developmental delay and macular ectopy. Two children (ZM16 and ZM27) had laboratory evidence of prenatal ZIKV infection, and one child (ZM17) was a twin brother of a child with ZBD and laboratory evidence of prenatal ZIKV infection (ZM18).

When last evaluated, 20/31 (65%) children had epilepsy, 22/31 (71%) had swallowing dysfunction, 15/30 (50%) had an eye abnormality, 7/29 (23%) had an abnormal hearing test, and 18/31 (58%) were underweight for age. Twenty-eight children (90%) had an abnormal neurodevelopmental assessment, of which, 21 (75%) had severe motor developmental delay and were only able to roll and/or lift their head in a prone position (Table 1).

Neuroimaging Findings

Twenty-nine children had adequate brain images and two were excluded due to only having skull radiography or head CT with bone window available. The median (range) child age at the neuroimaging study was 213 (3–939) days (Table 2). Five (17%) brain imaging studies were normal and belonged to normocephalic infants who had clinical findings of isolated left microphthalmia (ZM28), developmental delay and ASD (ZM05), developmental delay, and generalized hypotonia (ZM16), and evidence of maternal ZIKV disease but no neurological or neurodevelopmental compromise (ZM17 and ZM27, Supplemental Table). Twenty-four children had abnormalities on neuroimaging that included under-gyrification of the cerebral cortex (18/24, 75%), dysplastic corpus callosum (18/22, 82%), severe ventriculomegaly (15/24, 63%), and severe thinning of the cortical mantle (14/24, 58%) (Table 2). All 11 children with cerebral calcifications had them in the subcortical region; four children also had calcifications in the basal ganglia. Cerebral cysts or synechiae were in the frontal and/or temporal lobes (n=2) or the lateral ventricles (n=1).

Two-dimensional brain measurements

Twenty-four infants had images in DICOM format to measure cortical thickness in the frontal and occipital lobes (Table 3), of which three had normal brain findings and 21 had abnormal brain findings compatible with ZBD. The three infants with normal brain imaging

had a cortical thickness sum >10 cm. Twelve infants were classified in Pattern 1 with a combined sum of the right and left frontal and right and left occipital cortical thickness of <6 cm (represented by Figure 1 A-B); nine infants were classified in Pattern 2 with a combined cortical thickness of between 6–10 cm (represented by Figure 1 C-D). There was no difference in the infant age at the time of imaging between those with Pattern 1 and Pattern 2 (P = 0.1) (data not shown). The infants within Pattern 1 had a larger ventricular size and shorter corpus callosum length than infants in Pattern 2 (Table 3). Eight of 11 (73%) children with cerebral calcifications belonged to Pattern 1, including all four children with basal ganglia calcifications. All five children with occipital bone protuberance (Table 2) belonged to Pattern 1, while none of the children in Pattern 2 had this finding.

Comparative analysis of HC trajectories and clinical outcomes and characteristics between children in Pattern 1 and Pattern 2

Head circumference trajectories—HC trajectory for boys and girls within Pattern 1 and Pattern 2 is shown in Figure 2. The mean HC Z-score for measurements taken at the first evaluation was −3.14 for children within Pattern 1 and −1.37 for children within Pattern 2, and at the last evaluation was −7.19 for children within Pattern 1 and −4.71 for children within Pattern 2 (Figure 3). When compared, HC Z-score measurements for the first and last evaluations were significantly lower in children within Pattern 1 compared to in Pattern 2 (P<.05).

Clinical characteristics and outcomes—Selected clinical characteristics and outcomes for the 21 children classified in Pattern 1 and Pattern 2 is shown (Table 4). No differences were seen between age at last follow-up, GA at birth, trimester of maternal ZIKV symptoms and laboratory evidence of ZIKV infection between children with the two patterns. Among the clinical outcomes, abnormal eye findings were only seen in children with Pattern 1 and not in those in Pattern 2 (p<.01). Normocephaly at birth was more prevalent among children in Pattern 2 while microcephaly at birth was more prevalent among children in Pattern 1; however, this difference was not statistically significant (p=.07). There was a trend toward more advanced motor milestones among children in Pattern 2 compared with Pattern 1 (p=.06), where a higher proportion of children achieved the ability to sit, crawl, or walk. No correlations were established for outcomes such as underweight, swallowing dysfunction, epilepsy and abnormal hearing tests.

Discussion

We describe a cohort of children followed over two years from cities in Colombia that had a high prevalence of ZIKV disease in pregnant woman during the height of the 2015–2016 outbreak (Ospina, 2020). Most children in the cohort had microcephaly, severe neurologic morbidities, and significant cortical abnormalities consistent with ZBD on their clinical neuroimaging.

During follow-up to 19–42 months of age, most children had clinical manifestations related to congenital ZIKV infection – epilepsy, swallowing dysfunction, abnormal hearing, eye abnormalities, and profound motor developmental delay – increasing their disease severity and functional impairment. A high proportion of the children had epilepsy (65%). In other

ZBD cohorts, epilepsy is reported in 42–67% of children (Alves, 2018; Van Der Linden, 2018), and given the brain imaging severity in our cohort, the high burden of epilepsy was not unexpected. Although swallowing difficulties were clinically assessed, many caregivers reported that their child had issues with feeding starting at an early age. Because of feeding difficulties, over half of the children were underweight for age at follow-up. Leal et al. reported that feeding difficulties in children with ZBD usually present after the third month of life and may be as prevalent as 76% among severely affected children (Leal, 2017).

The 21 children with abnormal brain findings had imaging features ascribed to congenital ZIKV infection including thinning of the cortical mantle with under-gyrification, calcifications, dysplastic corpus callosum, ventriculomegaly, and occipital bone prominence (Moore, 2017). To separate the children by degree of severity, we performed 2D measurements of the right and left cortical mantle, which yielded two severity patterns of low cortical thickness. These patterns, which we termed Pattern 1 and Pattern 2, were based solely on the sum of cortical measurements and were performed independent of knowledge of the child's clinical outcomes and HC measurements. Children with Pattern 1 clearly had more advanced brain abnormalities which correlated with a higher burden of cerebral calcifications (8 of 12 children [67%]), were the only children to have calcifications in the basal ganglia (n=4), had a greater risk for ophthalmologic abnormalities, and showed a trend towards more severe neuromotor impairment. ZIKV has a tropism for immature neurons (Ho, 2017); it affects neuronal proliferation and causes neuronal apoptosis which is seen as a thin cortical mantle, under-gyrification, and microcephaly. The cortical measurements in our cases reflect the severity of neuronal injury and even though images occurred at different postnatal ages, the measurement patterns were distinct. Furthermore, there was no difference in the child's age at imaging between children with each pattern indicating that the measurements likely reflect the severity of arrested brain growth.

Similar to our recognition of different severities of cortical atrophy on imaging, Aragao et al. described different brain findings for infants with ZBD based on timing of microcephaly (Aragao, 2017). Infants with antenatal onset microcephaly had a simplified gyral pattern, and only infants with postnatal onset microcephaly had polymicrogyria. Calcifications outside of the cortical-subcortical white matter junction were found in children with microcephaly at birth which is similar to our cohort in which calcifications in the basal ganglia region were only present in the children within the more severe Pattern 1. In our cohort, all children with occipital bone protuberance were Pattern 1, which is consistent with severe prenatal onset microcephaly from disrupted brain growth (i.e., fetal brain disruption sequence). Almost 50% of children with imaging had postnatal-onset microcephaly. This proportion exceeds what has been reported in other studies and might reflect clinical findings that resulted in referral to our follow-up program. The imaging category was the less severe Pattern 2 for many of the children in our cohort with postnatal onset microcephaly. HC growth trajectory showed a continued failure of HC with further decline in HC Z-score over time among children with both patterns. The mean Z-score (<−7.0) seen in Pattern 1 reflects very compromised head growth that follows extensive damage to the developing brain. This level of head growth restriction and head growth deceleration has been previously described in other ZIKV cohorts (Prata-Barbosa, 2019; Ventura, 2016). Head size is related to brain volume and relates to neurodevelopmental outcomes (Wheeler,

2018). In our cohort, children with the more severely affected HC growth trajectory were in Pattern 1 where the measurement of cortical thickness was also severely reduced. Future larger studies should validate cortical thickness thresholds with specific outcomes.

A broad array of clinical, radiological, and developmental outcomes has been described in children affected with congenital ZIKV infection (Mulkey, 2019; Chimelli, 2017). Trimester of ZIKV infection has been identified as a risk factor for severe ZBD (Chimelli, 2017). Although our brain imaging patterns account for the extent of brain tissue damage seen from congenital ZIKV infection in this cohort (Tang, 2017), no differences between the patterns were seen for outcomes such us underweight, epilepsy, hearing abnormalities, or swallowing difficulties.

An analysis performed by Pool et al. of a ZBD cohort of 110 children in Brazil with neuroimaging showed that the presence of severe neurological compromise at birth and extensive structural brain abnormalities were more frequent when the infection was in the first trimester of pregnancy (Pool, 2019). Likewise, Mendes et al. proposed a classification of brain damage based on head CT findings in ZIKV exposed infants, establishing correlations between the most severe brain findings with trimester of ZIKV infection and severity of microcephaly at birth (Hoen, 2018). Thus, timing of ZIKV infection is a variable that has been consistently reported as being a determinant in the severity of clinical outcomes (Pool, 2019; Hoen, 2018; Karolina, 2020). However, because there are few infections outside of first trimester in our cohort, differences by trimester of infection were not able to be assessed. Other potential factors that may influence clinical findings include ZIKV viral load, past maternal exposure to other flaviviruses, maternal-fetal immunologic responses, and an individuaĺs own genetic background (Caires-Júnior, 2018).

Abnormal funduscopic examination in congenital ZIKV infection frequently accompanies brain abnormalities and is found in 21–55% of ZIKV cohorts (Ventura, 2018). ZIKV can directly affect the retinal pigment epithelium, choroid vessels, and optic nerve (Fernandez, 2017). Previous reports related ZIKV brain abnormalities and neurologic alterations as being most frequent when there is ocular compromise (Cranston, 2020; Pool, 2019), suggesting that structural eye abnormalities may be a hallmark of a more severe disease pattern. Ophthalmological findings without other clinical compromise have been reported to occur between 3–7% in other ZBD cohorts (Tsui, 2018; Zin, 2017). These findings highlight the importance of performing thorough clinical evaluations including ophthalmological assessment in all children with antenatal ZIKV exposure, even in the absence of more severe disease.

Although child neurodevelopment was not assessed by a standardized evaluation, milestone tracking during the visits allowed the identification of motor developmental impairments. At the last evaluation, most children had severe neuromotor compromise. Like our cohort, most case series report a profound developmental delay in language, motor, and social domains (Alves, 2018). The structural and functional brain abnormalities found in children with congenital ZIKV infection, have been related to the severity of neurological and neurodevelopmental alterations (Wheeler, 2018; Hcini, 2021), and as expected, children with the most severe brain compromise (Pattern 1 in our cohort), tended to have poorer motor

outcomes. To date, no publications have correlated 2D neuroimaging measurement patterns to motor or other developmental outcomes for children with congenital ZIKV infection, highlighting the importance of further studies in this area.

A few children presented for evaluation by INS because of clinical concerns of hypotonia and developmental delay; however, neuroimaging was not consistent with sequelae related to ZBD. Despite the probable *in utero* exposure to ZIKV, their clinical neurologic abnormalities were thought to relate to other conditions and not to ZIKV exposure, although this cannot be completely ruled out. Clinicians caring for children in regions of endemic ZIKV exposure should consider the range of etiologies for child neurodevelopmental delay.

Limitations

Our study is strengthened by the correlation of clinical neuroimaging to longitudinal follow-up but also has several limitations. Children were recruited from the national ZIKV disease and birth defects surveillance systems, where symptomatic pregnant women and infants with possible ZBD were monitored regardless of maternal or infant laboratory evidence of ZIKV infection and thus should be considered as a convenience sample subject to inherent potential biases. Although all children were assessed by expert clinicians, who assigned a possible ZBD diagnosis based on clinical findings, some could be misclassified as having etiologies explaining clinical findings other than congenital ZIKV infection. All children were assessed by neuropediatricians who tracked the attainment of developmental milestones, but no standardized instruments were used in our evaluations and only motor milestones were included in this analysis. Not all children evaluated had brain MRIs and some had limited sequences or images were of inadequate quality for review. There was variability in the plane of imaging in the cases which may have affected cortical measurements. We did not measure other brain structures such as the cerebellar vermis and brainstem due to variation in images which may be important for outcomes such as arthrogryposis. Also, since the clinical follow-up was done as part of ZIKV surveillance, swallow studies, hearing tests, and neuroimaging were taken at different time points and by different modalities.

Conclusion

In children with ZBD, we found that less cortical thickness correlated with ophthalmologic outcomes and functionality. The imaging patterns relate to HC growth trajectory, even for infants whose HC was classified as normocephalic at birth and fell into the microcephalic range over time. Based on these findings, 2D measurements of cerebral cortical thickness may be a useful marker for the severity of future clinical outcomes in children with brain abnormalities from congenital ZIKV infection. This type of measurement can be performed with readily available software and may aid in the evaluation of other ZBD cohorts. Further studies are needed to explore the relationship between neuroimaging findings and the range of clinical outcomes and functionality in children with ZBD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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CDC Disclaimer:

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Data Availability Statement:

Restrictions apply to the availability of these data. Specific data may be available upon request to the corresponding author.

References

Alves L V, Mello MJG, Bezerra PG, Alves JGB. Congenital Zika Syndrome and infantile spasms: case series study. J Child Neurol 2018;33:664–6. 10.1177/0883073818780105. [PubMed: 29897010]

Alves L V, Paredes CE, Silva GC, Mello, Alves JG. Neurodevelopment of 24 children born in Brazil with congenital Zika syndrome in 2015: A case series study. BMJ Open 2018;8. 10.1136/bmjopen-2017-021304.

Aragao MFVV Holanda AC, Brainer-Lima AM, Petribu NCL, Castillo M, Van Der Linden V, et al. Nonmicrocephalic infants with congenital zika syndrome suspected only after neuroimaging evaluation compared with those with microcephaly at birth and postnatally: How large is the Zika virus "iceberg"? Am J Neuroradiol 2017;38:1427–34. 10.3174/ajnr.A5216. [PubMed: 28522665]

Ashwal S, Michelson D, Plawner L, Dobyns WB, Quality Standards Subcommittee of the American Academy of N, the Practice Committee of the Child Neurology S. Practice parameter: Evaluation of the child with microcephaly (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Neurology. 2009;73(11):887–97. doi: 10.1212/WNL.0b013e3181b783f7. PubMed PMID: 19752457; PMCID: PMC2744281. [PubMed: 19752457]

Caires-Júnior LC, Goulart E, Melo US, Araujo BSH, Alvizi L, Soares-Schanoski A, et al. Discordant congenital Zika syndrome twins show differential in vitro viral susceptibility of neural progenitor cells. Nat Commun 2018;9:1–11. 10.1038/s41467-017-02790-9. [PubMed: 29317637]

Chimelli L, Melo ASO, Avvad-Portari E, Wiley CA, Camacho AHS, Lopes VS, et al. The spectrum of neuropathological changes associated with congenital Zika virus infection. Acta Neuropathol 2017;133:983–99. 10.1007/s00401-017-1699-5. [PubMed: 28332092]

Cranston JS, Tiene SF, Nielsen-Saines K, Vasconcelos Z, Pone MV, Pone S, et al. Association between antenatal exposure to Zika virus and anatomical and neurodevelopmental abnormalities in children. JAMA Netw Open 2020;3:e209303. 10.1001/jamanetworkopen.2020.9303.

Cuevas Ortiz EL, Martínez Ospina ML, Martinez Duran ME. Protocolo de vigilancia en salud pública: vigilancia intensificada en salud pública de la microcefalia y otros defectos congénitos del sistema nervioso central 2016:26.

De Onis M. 4.1 The WHO child growth standards. World Rev Nutr Diet 2015;113:278–94. 10.1159/000360352. [PubMed: 25906897]

Fernandez MP, Parra Saad E, Ospina Martinez M, Corchuelo S, Mercado Reyes M, Herrera MJ, et al. Ocular histopathologic features of congenital Zika syndrome. JAMA Ophthalmol 2017;135:1163–9. 10.1001/jamaophthalmol.2017.3595. [PubMed: 28975230]

Galang RR, Avila GA, Valencia D, Daza M, Tong VT, Bermúdez AJ, et al. Etiology of microcephaly and central nervous system defects during the Zika epidemic in Colombia. J Pediatr 2020; 222: 112–119. 10.1016/j.jpeds.2020.02.023. [PubMed: 32417080]

Hcini N, Kugbe Y, Rafalimanana ZHL, Lambert V, Mathieu M, Carles G, et al. Association between confirmed congenital Zika infection at birth and outcomes up to 3 years of life. Nat Commun 2021;12, 3270 (2021). 10.1038/s41467-021-23468-3

Ho CY, Ames HM, Tipton A, Vezina G, Liu JS, Scafidi J, et al. Differential neuronal susceptibility and apoptosis in congenital Zika virus infection. Ann Neurol 2017;82:121–7. 10.1002/ana.24968. [PubMed: 28556287]

Hoen B, Schaub B, Funk AL, Ardillon V, Boullard M, Cabié A, et al. Pregnancy outcomes after ZIKV infection in French territories in the Americas. N Engl J Med 2018;378:985–94. 10.1056/NEJMoa1709481. [PubMed: 29539287]

Instituto Nacional de Salud. Public Health Surveillance Protocol: Zika Virus Disease, Código 895. Available at: https://www.ins.gov.co/buscadoreventos/Lineamientos/PRO%20Zika.pdf.

Karolina A, Mendes T, Rodrigues M, Ribeiro C, Lamy-filho F, Amaral GA, et al. Congenital Zika Syndrome: Association between the gestational trimester of maternal infection, severity of brain computed tomography findings and microcephaly at birth. Revista do Instituto de Medicina Tropical de Sao Paulo2020:1–8. 10.1590/S1678-9946202062056.

Leal MC, van der Linden V, Bezerra TP, de Valois L, Borges ACG, Antunes MMC, et al. Characteristics of dysphagia in infants with microcephaly caused by congenital zika virus infection, Brazil, 2015. Emerg Infect Dis 2017;23:1253–9. 10.3201/eid2308.170354. [PubMed: 28604336]

Lima DLP, Correia MLGCD, Monteiro MG, Ferraz KM, Wiesiolek CC. Análise do desempenho funcional de lactentes com síndrome congênita do zika: estudo longitudinal. Fisioter e Pesqui 2019;26:145–50. 10.1590/1809-2950/18001626022000b519.

Moore CA, Staples JE, Dobyns WB, Pessoa A, Ventura CV, Da Fonseca EB, et al. Characterizing the pattern of anomalies in congenital zika syndrome for pediatric clinicians. JAMA Pediatr 2017;171:288–95. 10.1001/jamapediatrics.2016.3982. [PubMed: 27812690]

Mulkey SB, Arroyave-Wessel M, Peyton C, Bulas DI, Fourzali Y, Jiang J, et al. Neurodevelopmental abnormalities in children with in utero Zika virus exposure without Congenital Zika Syndrome. JAMA Pediatr 2020;174:269–76. 10.1001/jamapediatrics.2019.5204. [PubMed: 31904798]

Mulkey SB. Importance of neuroimaging in the evaluation of Zika virus-exposed infants. JAMA Netw Open 2019;2:e198137. 10.1001/jamanetworkopen.2019.8137.

Nielsen-Saines K, Angeles L, De Janeiro R, Kerin T, Angeles L, Vasconcelos Z, et al. Delayed childhood neurodevelopment and neurosensory alterations in the second year of life in a prospective cohort of ZIKV exposed children. Nat Med 2019;25:1213–7. 10.1038/s41591-019-0496-1. [PubMed: 31285631]

Olson SM, Delaney A, Jones AM, Carr CP, Liberman RF, Forestieri NE, et al. Updated baseline prevalence of birth defects potentially related to Zika virus infection. Birth Defects Res 2019;111:938–40. 10.1002/bdr2.1546. [PubMed: 31264801]

Ospina ML, Tong VT, Gonzalez M, Valencia D, Mercado M, Gilboa SM, et al. Zika virus disease and pregnancy outcomes in Colombia. N Engl J Med 2020;383:537–45. 10.1056/nejmoa1911023. [PubMed: 32757522]

Pereira HVFS, dos Santos SP, Amâncio APRL, de Oliveira-Szejnfeld PS, Flor EO, de Sales Tavares J, et al. Neurological outcomes of congenital Zika syndrome in toddlers and preschoolers: a case series. Lancet Child Adolesc Heal 2020;4:378–87. 10.1016/S2352-4642(20)30041-9.

Pool KL, Adachi K, Karnezis S, Salamon N, Romero T, Nielsen-Saines K, et al. Association between neonatal neuroimaging and clinical outcomes in Zika-exposed infants from Rio de Janeiro, Brazil. JAMA Netw Open 2019;2:1–14. 10.1001/jamanetworkopen.2019.8124.

Prata-Barbosa A, Martins MM, Guastavino AB, Cunha AJLA da. Effects of Zika infection on growth. J Pediatr (Rio J) 2019;95:30–41. 10.1016/j.jped.2018.10.016. [PubMed: 30593788]

de Colombia República. Ministerio de la Protección social. Decreto número 2323 de 2006, por el cual se reglamenta parcialmente la ley 09 de 1979, en relación con la Red Nacional

de Laboratorios. Available at: https://www.invima.gov.co/images/pdf/red-nal-laboratorios/decretos/DECRETO_2323_DE_2006.pdf.

de Colombia República. de Salud Pública Ministerio. Decreto número 786 de 1990 por el cual se reglamenta el título IX de la ley 09 de 1979, en cuanto a la práctica de autopsias clínicas y médico-legales, así como viscerotomías y se dictan otras disposiciones. Available at: http://www.suinjuriscol.gov.co/viewDocument.asp?id=1165673.

Tang H, Hammack C, Ogden SC, Wen Z, Qian X. et al. Zika virus infects human cortical neurarl precursos and attenuates their growth. Cell stem cell 2017;18:587–90. 10.1016/j.stem.2016.02.016.Zika.

Tsui I, Moreira MEL, Rossetto JD, et al. Eye findings in infants with suspected or confirmed antenatal Zika virus exposure. Pediatrics. 2018;142(4):e20181104. 10.1542/peds.2018-1104.

Van Der Linden H, Carvalho MD, Van Der Linden V, Lacerda KM, Pessoa A, Carneiro ML, et al. Epilepsy profile in infants with congenital zika virus infection. N Engl J Med 2018;379:891–2. 10.1056/NEJMc1716070. [PubMed: 30157396]

Ventura CV, Maia M, Dias N, Ventura LO, Jr RB. Zika : neurological and ocular fi ndings in infant without microcephaly. Lancet 2016;387:2502. 10.1016/S0140-6736(16)30776-0. [PubMed: 27287830]

Ventura CV, Ventura LO. Ophthalmologic manifestations associated with Zika virus infection. Pediatrics 2018;141;S161. 10.1542/peds.2017-2038E.

Villar J, Ismail LC, Victora CG, Ohuma EO, Bertino E, Altman DG, et al. International standards for newborn weight, length, and head circumference by gestational age and sex: The newborn cross-sectional study of the INTERGROWTH-21st Project. Lancet 2014;384:857–68. 10.1016/s0140-6736(14)60932-6. [PubMed: 25209487]

Wheeler AC, Ventura CV, Ridenour T, Toth D, Nobrega LL, de Souza Dantas LCS, et al. Skills attained by infants with congenital Zika syndrome: Pilot data from Brazil. PLoS One 2018;13:1–9. 10.1371/journal.pone.0201495.

Wheeler AC. Development of infants with congenital zika syndrome: What do we know and what can we expect? Pediatrics 2018;141:S154–60. 10.1542/peds.2017-2038D. [PubMed: 29437048]

Zin AA, Tsui I, Rossetto J, Vasconcelos Z, Adachi K, Valderramos S, et al. Screening criteria for ophthalmic manifestations of congenital zika virus infection. JAMA Pediatr 2017;171:847–54. 10.1001/jamapediatrics.2017.1474. [PubMed: 28715527]

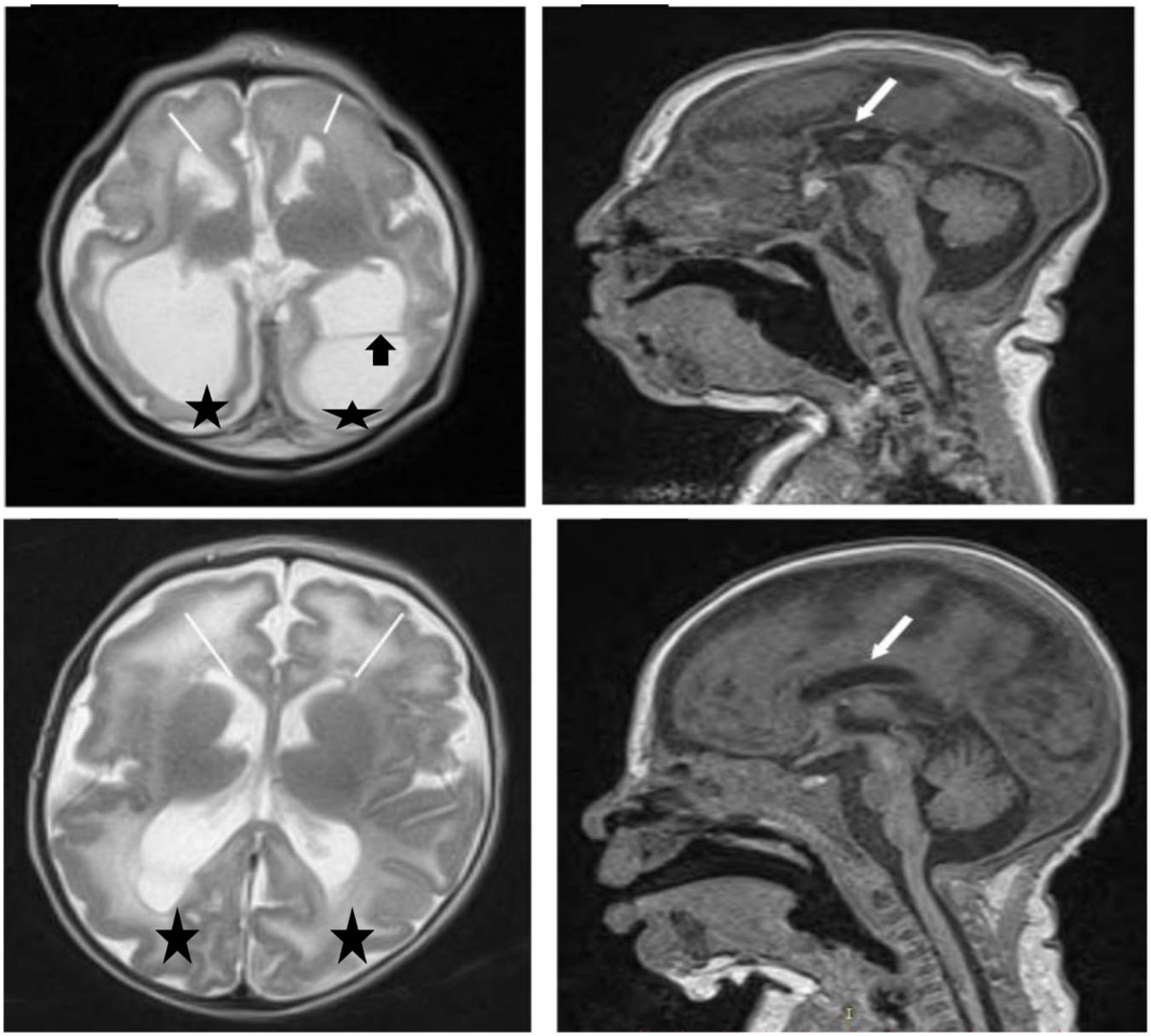
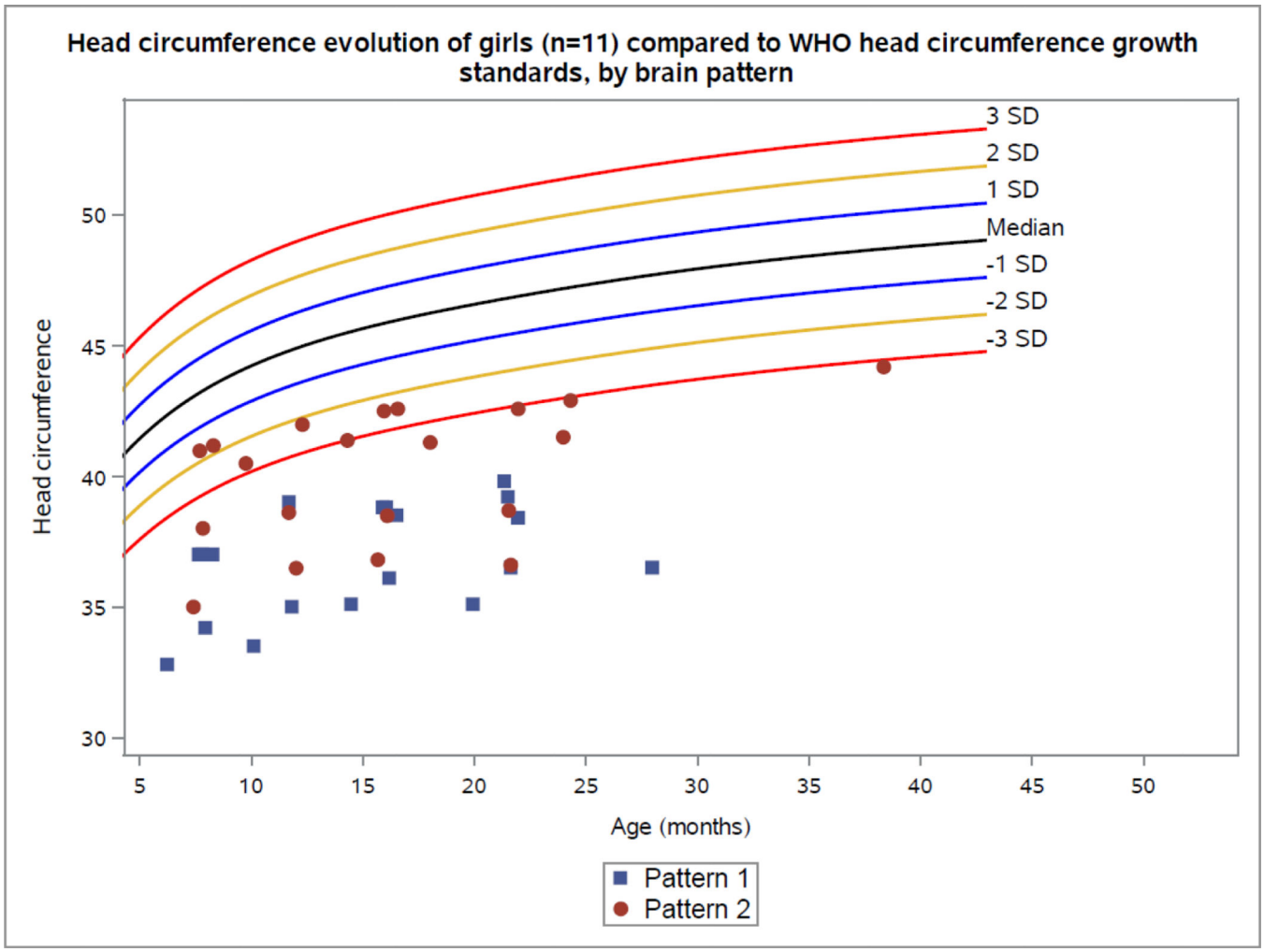


Figure 1.

Representative Brain MRI patterns in two infants with microcephaly attributable to probable *in utero* ZIKV exposure



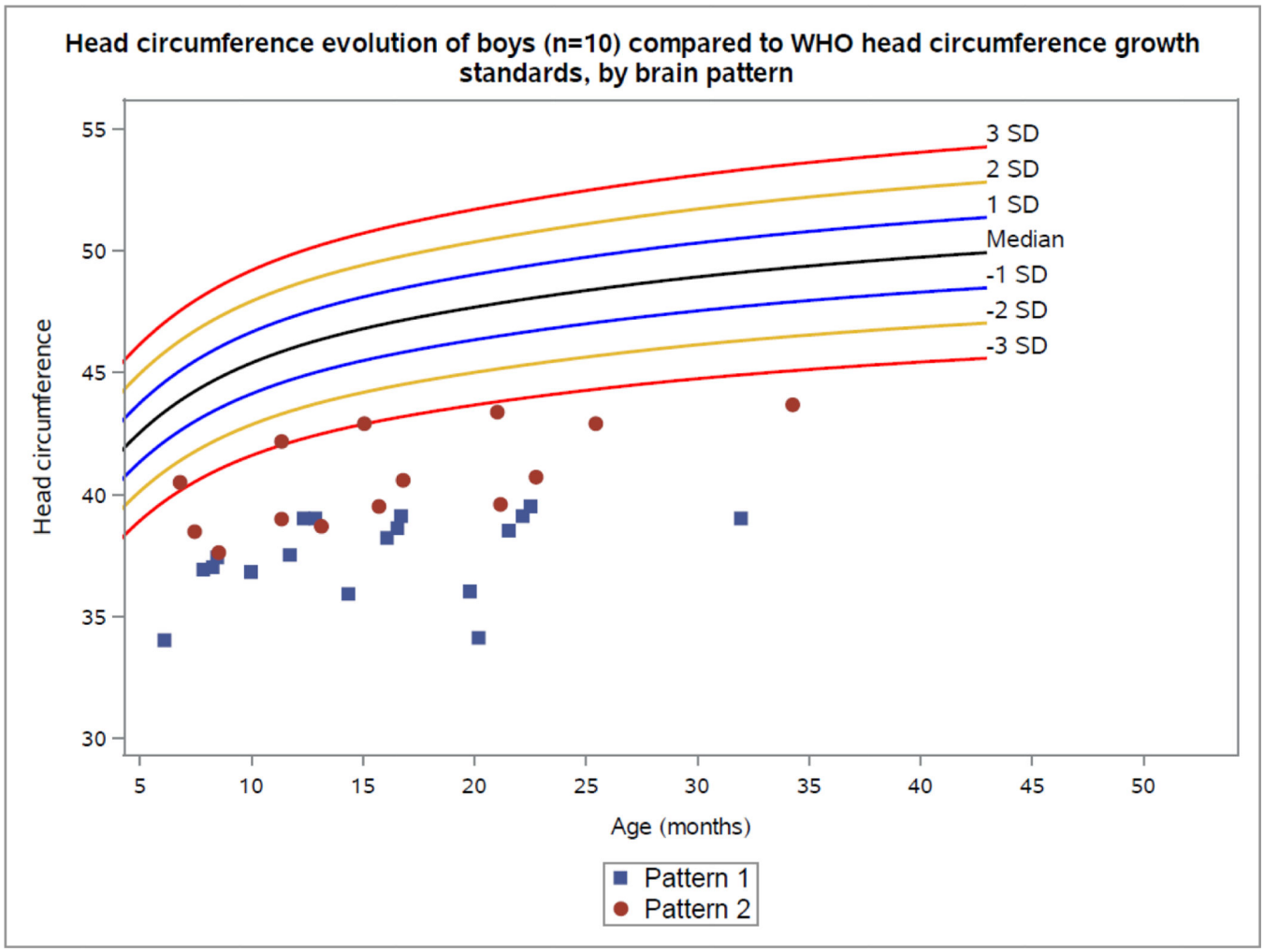


Figure 2.

Plot of head circumference standard deviation by age of the child (in months), sex, and brain pattern – cohort of children with probable *in utero* ZIKV exposure*

*Head circumference at birth was excluded in the figure because INTERGROWTH 21st Newborn Size Standards and References was used for all infants at birth

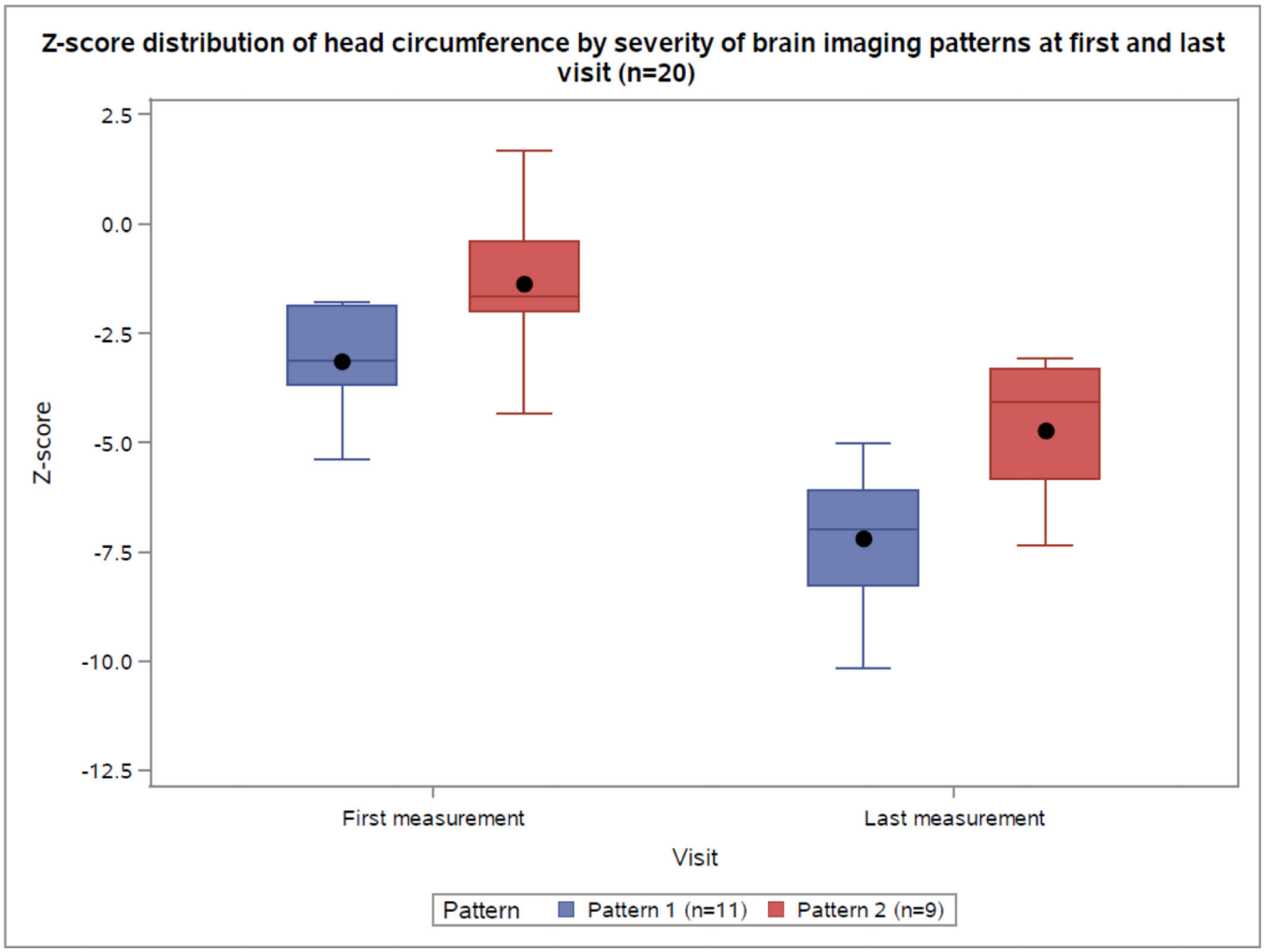


Figure 3.

Z-score distribution of head circumference by neuroimaging pattern at first and last clinical visits – cohort of children with probable *in utero* ZIKV exposure

Boxplots of children's head circumference Z-scores for the HC measurement at the first visit and at the last visit. Pattern 1 boxplots are shown in blue and pattern 2 are in red.

*One child with pattern 1 was excluded due to only having one HC measurement.

**Using the Wilcoxon Rank Sum test, Z-score were significantly lower for children with the most severe brain pattern at both visits ($p<.05$).

***The circle in the box represents the group mean, and the horizontal line in the box represents the group median.

Table 1.

Selected Maternal and Infant Characteristics for Cohort of Children with probable *in utero* ZIKV Exposure (N=31)

Characteristic	N (column %)
Maternal age at delivery, years (median [range])	23 (17–34)
Trimester of maternal ZIKV symptoms	
First	21 (68)
Second	4 (13) ¶
Third	1 (3)
Unknown trimester	1 (3)
No symptoms	4 (13)
Laboratory evidence of ZIKV infection in mother or child *	
Yes	10 (32)
No	8 (26)
No testing data available	13 (42)
Gestational age at birth (weeks)	
≤31	1 (3)
32–36	4 (13)
≥37	26 (84)
Child sex	
Male	17 (55)
Female	14 (45)
Microcephaly at birth †**(n=26)**	12 (46)
Small for gestational age ‡**(n=30)**	5 (17)
Clinical outcomes at last visit	
Microcephaly §§	26 (84)
Epilepsy	20 (65)
Swallowing dysfunction	22 (71)
Underweight for age&	18 (58)
Abnormal hearing test (n=29)	7 (24)
Abnormal eye findings (n=30)	15 (50)
Abnormal neurologic or neurodevelopmental assessment	28 (90)
Most advanced motor milestone at last visit (n=28)¶¶	
Ambulation with or without assistance	4 (14)
Crawling and/or Sitting with or without support	3 (11)
Rolls and/or lifts head when prone	21 (75)
Radiography available	
Brain MRI	22 (71)

Characteristic	N (column %)
Head CT	12 (39)
Skull/head X-ray	2 (6)
Swallow study	6 (19)
Other X-rays	12 (39)

Abbreviations: CT=cranial tomography, HC=head circumference, MRI=magnetic resonance imaging, SD=standard deviation, ZIKV=Zika virus

¶Three children of asymptomatic mother but symptomatic father

*Either positive for ZIKV using real-time reverse transcriptase polymerase chain reaction or positive for ZIKV immunoglobulin-M in mother or child

+HC for gestational age and sex below −2 SD (Intergrowth 21st - Neonatal growth standards)

++HC for age and sex below −2 SD (WHO growth standards)

**Birth weight for gestational age and sex below the 10th percentile (Intergrowth 21st - Neonatal growth standards)

¶¶For children with abnormal neurologic or neurodevelopmental assessment. Median age at last visit 22 months (range 19–42 months).

&Weight for age and sex below< −2 SD (WHO growth standards)

Table 2.

Summary of Neuroimaging Findings for Cohort of Children with probable *in utero* ZIKV Exposure

Brain Imaging	**Children (n=29)**[¶] (column %)
Days of age at brain imaging (MRI and/or CT) (median, range)	213 (3–939)
Brain MRI	23 (79)
Head CT	12 (41)
Both MRI and CT	6 (21)
Overall Brain Findings	
Normal	5 (17)
Abnormal	24 (83)
Specific Imaging Features in Abnormal Scans	**Children (n=24)** [*] (column %)
Calcifications	11 (46)
Thin corpus callosum (n=22)	18 (82)
Severe ventriculomegaly	15 (63)
Severe thinning of cortical mantle	14 (58)
Under-gyrification of cortex	18 (75)
Severe cerebellar hypoplasia (n=21)	1 (4)
Occipital bone protuberance/shelf (n=20)	5 (25)
Cerebral cyst(s) or synechiae	3 (13)

Abbreviations: CT=cranial tomography, MRI=magnetic resonance imaging

[¶]Two children from the data set of 31 were not included due to only skull radiography or bone head CT images available.

[*]For all, n=24, except where noted otherwise. In some cases, “n” differs owing to inadequate MRI sequences to comment on calcifications, corpus callosum, or occipital bone shape.

Table 3:

Two-dimensional brain MRI measurements for the cohort of children with probable *in utero* ZIKV exposure with normal, pattern 1 and pattern 2 brain imaging findings (n=24)*

Brain structure ¶	Normal (n=3) (cm) (mean and SD)	Pattern 1 (n=12) (cm) (mean and SD)	Pattern 2 (n=9) (cm) (mean and SD)
Right frontal cortex	3.1 (1.0)	1.1 (0.3)	2.1 (0.4)
Left frontal cortex	2.8 (0.8)	1.1 (0.4)	2.1 (0.4)
Right occipital cortex	2.9 (0.6)	0.7 (0.3)	1.5 (0.5)
Left occipital cortex	2.8 (0.5)	0.6 (0.2)	1.7 (0.4)
Right ventricle	0.9 (0.1)	2.1 (0.9)	1.5 (0.8)
Left ventricle	0.9 (0.1)	2.3 (0.8)	1.7 (1.1)
Corpus callosum	5.6 (1.4)	2.6 (1.5)	4.2 (1.3)
Cortical thickness sum	13.0 (1.0)	3.4 (1.0)	7.4 (1.2)

Abbreviations: cm: centimeter SD= standard deviation MRI=magnetic resonance imaging.

*24 of 29 children with neuroimaging had DICOM format, adequate sequences, and planes of imaging for measurement.

¶Measurements were done on DICOM images and are not adjusted for age at image acquisition. Three children in pattern 1 had only head CT, all other children had brain MRI for measurement.

Normal brain images include studies with no structural brain abnormalities and a cortical thickness sum above 10 cm. Pattern 1 was defined when the cortical thickness sum was below 6 cm. Pattern 2 was defined when the cortical thickness sum was between 6–10 cm.

Table 4.

Selected clinical characteristics and outcomes by neuroimaging groups – cohort of children with probable *in utero* ZIKV exposure

		Pattern 1 (n=12) N (%)	Pattern 2 (n=9) N (%)
Age at last follow-up (months)	19–25	10 (83)	7 (78)
	26–38	2 (17)	2 (22)
Gestational age at delivery (weeks)	<37	3 (25)	1 (11.1)
	≥37	9 (75)	8 (89)
Trimester of maternal ZIKV symptoms	First	10 (83)	6 (67)
	Second	1 (8)	2 (22)
	Missing	1 (8)	1 (11)
Laboratory evidence of ZIKV infection in mother or child		5 (56)	3 (43)
Missing laboratory results for ZIKV infection		3 (25)	2 (22)
HC at birth (Z-score for GA and sex)	≥ - 2.0 SD	3 (27)	7 (78)
	<−2.0 ≥ - 3.0 SD	2 (18)	1 (11)
	< −3.0 ≥ −7.0 ** SD	6 (55)	1 (11)
	< −7.0 SD	0 (0)	0 (0)
	Missing	1 (8)	0 (0)
HC at last evaluation (Z-score for age and sex)	≥ - 2.0 SD	0 (0)	0 (0)
	<−2.0 ≥ - 3.0 SD	0 (0)	0 (0)
	< −3.0 ≥ −7.0 ** SD	6 (50)	8 (89)
	< −7.0 SD	6 (50)	1 (11)
Underweight for age at last evaluation +		8 (67)	5 (56)
Symptoms suggestive of swallowing difficulties		11 (92)	6 (67)
Epilepsy at last evaluation		10 (83)	5 (56)
Abnormal eye findings		9 (75)	0 (0)
Motor milestones	Ambulation with or without assistance	1 (8)	1 (11)
	Crawling and/or Sitting with or without support	0 (0)	3 (33)
	Rolls and/or lifts head when prone	11 (92)	5 (56)
Abnormal hearing test		3 (27)	2 (22)
	Missing	1 (8)	0 (0)

Abbreviations: GA= gestation age, HC= head circumference, SD=standard deviations, ZIKV=Zika virus.

** A cutoff of −7 SD was established to compare Z-score differences within the two patterns, based on the mean Z-score of HC measurements at the last evaluation for children in Pattern 1.

+ Weight for age and sex below< −2 SD (WHO growth standards)

Note: the number of children included in this analysis is not enough to perform a sensitivity analysis