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ORIGINAL ARTICLE

Zika virus congenital microcephaly severity classification and the association of severity with neuropsychomotor development

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Abstract

Background Zika virus infection during pregnancy is linked to birth defects, most notably microcephaly, which is associated with neurodevelopmental delays.

Objective The goals of the study were to propose a method for severity classification of congenital microcephaly based on neuroradiologic findings of MRI scans, and to investigate the association of severity with neuropsychomotor developmental scores. We also propose a semi-automated method for MRI-based severity classification of microcephaly.

Materials and methods We conducted a cross-sectional investigation of 42 infants born with congenital Zika infection. Bayley Scales of Infant and Toddler Development III (Bayley-III) developmental evaluations and MRI scans were carried out at ages 13–39 months (mean: 24.8 months; standard deviation [SD]: 5.8 months). The severity score was generated based on neuroradiologist evaluations of brain malformations. Next, we established a distribution of Zika virus–microcephaly severity score including mild, moderate and severe and investigated the association of severity with neuropsychomotor developmental scores. Finally, we propose a simplified semi-automated procedure for estimating the severity score based only on volumetric measures.

Results The results showed a correlation of r=0.89 (P<0.001) between the Zika virus–microcephaly severity score and the semi-automated method. The trimester of infection did not correlate with the semi-automated method. Neuropsychomotor development correlated with the severity classification based on the radiologic readings and semi-automated method; the more severe the imaging scores, the lower the neuropsychomotor developmental scores.

Conclusion These severity classification methods can be used to evaluate severity of microcephaly and possible association with developmental consequences. The semi-automated methods thus provide an alternative for predicting severity of microcephaly based on only one MRI sequence.

Keywords Brain · Congenital microcephaly · Development · Infants · Magnetic resonance imaging · Zika virus

Introduction

In 2015, an epidemic of Zika virus infection affected Brazil, especially the northeastern region of the country. Between March 2015 and February 2016, there was a 20-fold increase in births with microcephaly in Brazil as compared to an equivalent previous time period [1]. Newborns of mothers infected with Zika virus during pregnancy presented with severe brain malformations and abnormalities of development [2], most notably microcephaly [3, 4]. The identification of Zika virus ribonucleic acid (RNA) in the amniotic fluid of mothers whose fetuses had cerebral abnormalities suggested that Zika virus transmission occurred during pregnancy [5, 6]. Zika-virus-related microcephaly is associated with central nervous system lesions that include destruction, calcification, hypoplasia and migration disturbances [7]. Moreover, studies show that the brain is susceptible to the effects of Zika virus infection in multiple developmental stages [8].

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The developmental outcomes associated with microcephaly (e.g., non-Zika-virus-related) vary. One study showed that 50% of children with mild microcephaly had normal intelligence scores [9]. The severity of Zika-virus-related microcephaly, however, has been associated with severity of cognitive [10] and motor [11] impairments. In this sense, a combination of developmental and brain imaging evaluations might help predict and better characterize microcephaly-related outcomes, especially in the more recent Zika-virus-related microcephaly; a system of evaluation and scoring might help clinicians understand and evaluate, with reproducibility, Zika-virus-related microcephaly effects and allow for comparison to other cases of microcephaly.

The goal of the present study was to produce a score for the severity of microcephaly using a combination of clinical and brain imaging indices. Previous studies have investigated the relationship between brain imaging of Zika-virus-related microcephaly and development [12]. But, to our knowledge, no studies have proposed a brain-imaging-based classification of Zika-virus-related microcephaly. We further investigated the image characteristics that were more relevant for the classification, and we established a semi-automatic algorithm to predict the severity score using only the T1-weighted sequence. Last, we investigated the relation between the severity score and the motor, language and cognitive development evaluations.

Materials and methods

Participants

The study included 42 infants born with suspected or confirmed congenital Zika virus (19 females; mean age: 24.8 months, standard deviation [SD]: 5.8 months; head circumference ≤32 cm) who were registered at a state health department. Inclusion criteria for mothers and infants followed Brazilian Ministry of Health guidelines [13]. The criteria for including mothers were history of pruritic maculopapular exanthema, positive Zika virus immunoglobulin M serological reaction, and at least two Zika virus infection symptoms. Inclusion criteria for infants were being positive for Zika virus immunoglobulin G and born to mothers with suspected or confirmed Zika virus infection during pregnancy. Mothers and infants were screened for the following congenital infectious disorders, which represented exclusion criteria: syphilis, toxoplasmosis, rubella, cytomegalovirus and herpes simplex. No infant tested positive for any of these infections. We obtained written informed consent from the parents or guardians of the infants. The study was approved by the research ethics committee of the Pontifical Catholic University of Rio Grande do Sul.

Instruments and procedures

We collected Bayley Scales of Infant and Toddler Development (Bayley-III) 1 day after the MRI exam. We used the Brazilian Portuguese version of the Bayley-III [14] scales to assess three domains: cognition, language (receptive and expressive communication) and motor skills (gross and fine). Head circumference was measured at birth and at the MRI exam; we also generated a head circumference growth ratio score by subtracting head circumference at birth from head circumference on the day of the MRI exam and dividing the result by head circumference at birth.

Magnetic resonance imaging data acquisition

Twenty-eight infants were scanned at the Brain Institute of Rio Grande do Sul in Porto Alegre, Rio Grande do Sul, and 14 were scanned at the Memorial Hospital Arthur Ramos in Maceió, Alagoas; see Online Supplementary Material 1 for a detailed description of the sequence parameters. To avoid head motion artifacts during MRI scans, participants at both sites were anesthetized. The MRI exams were carried out under the supervision of an expert anesthesiologist.

Zika virus–microcephaly severity score

Brain MR images were analyzed by two neuroradiologists (R.B.S. and R.C.B., each with 15 years of experience). They performed independent analyses of the MR images. The neuroradiologists were aware they were evaluating Zika-virus-related microcephaly cases, but they were blinded to the medical history and clinical information about the infant; they were also blinded to each other's evaluation. After the neuroradiologic evaluations were performed, disagreements in evaluations were resolved by consensus in a case-by-case discussion.

The neuroradiologists reviewed the brain MR images to investigate structural abnormalities based on 13 characteristics. The imaging characteristics were drawn from studies of brain effects of congenital microcephaly in babies born during the Zika virus outbreak in Brazil [8, 15–17]. Except for calcifications, the characteristics were scored on a 4-point scale from 0 to 3, with 0 representing normality and 3 the most severe abnormality according to the neuroradiologist. The brain calcifications were scored on a 5-point scale from 0 to 4, with 0 representing absence of calcifications and 4 the presence of calcifications in four or more regions. The brain regions analyzed for the calcifications were the cortico-subcortical white matter junction, periventricular region, basal ganglia and posterior fossa. We used five imaging sequences to evaluate the imaging characteristics.

The specific imaging sequence employed for each score is described in the Online Supplementary Material 2. There were disagreements between the neuroradiologists on 24 of 546 (4%) characteristics.

The Zika virus–microcephaly severity score grouping was established using the scores of the 13 malformations of the different MRI structural scans. The three categories were established dividing the total score into terciles: severity scores ranging from 0 to 12 were classified as mild microcephaly; from 12 to 25, moderate microcephaly; and above 26, severe microcephaly. The maximum score was 41.

We present a method to semi-automatically create the Zika virus–microcephaly severity score and establish severity of microcephaly-related abnormalities. The motivation to create this semi-automated method was to remove the subjectivity of radiologic findings and thus establish reproducibility for the severity score. The method is based on MRI volumetric measures alone. The goal was to predict the severity score using only one brain imaging sequence (T1 structural scan) that delineates four volumes of interest (VOI) for each infant. The volumes were: (a) the lateral ventricles, (b) whole brain, (c) intracranial and (d) the cerebellum segmentation (see Online Supplementary Material 3). We applied a semi-automated region-growing segmentation method based on the edge-detection algorithm in the Insight Segmentation and Registration Toolkit software [18]. The regression model and the procedure for segmentation of the VOIs are described in Online Supplementary Material 4. This procedure allows for replication of evaluation across sites and research of microcephaly-related developmental outcomes independent of neuroradiologic evaluations. The goal, of course, is not to replace neuroradiologic evaluation but rather to afford an instrument for research purposes.

Statistical analysis

We carried out statistical analyses using the Statistical Package for Social Sciences (SPSS Statistics version 23; IBM, Armonk, NY) (RRID:SCR_019096). We used descriptive statistics for the population demographics. We used the Kolmogorov-Smirnov nonparametric test to evaluate whether clinical variables (age, head circumference at birth and on day of MRI exam, and trimester of infection) and volumetric variables had a normal distribution. The Kolmogorov-Smirnov test showed that all variables had a non-parametric distribution. Next, we applied the Spearman correlation to investigate the relationship among variables. We used partial correlations and controlled for age for the analyses of Bayley-III scales and the clinical and imaging variables. We used the raw score of Bayley-III to perform analysis of correlations among cognitive, receptive and expressive communication, and fine and gross motor scores with the imaging readings scores. We calculated the alpha coefficient of Cronbach to verify the internal consistency, measuring the reliability of the radiologic severity score. We performed a linear regression analysis to create the semi-automated Zika virus–microcephaly severity score (dependent variable) by using the intracranial volume (x_1), the ratio between lateral ventricles and brain volume (x_2), the ratio between brain and intracranial volume (x_3), and the square of each one of the three volumetric variables (x_1^2, x_2^2, x_3^2). $P<0.05$ was considered statistically significant for all analyses.

Results

Participant demographics

We evaluated 42 infants with confirmed Zika virus infection during gestation. The participants' mean birth weight was 2,650 g (SD: 0.53 g); 37 participants were born at full term. The mean head circumference (HC) at birth was 29.8 cm (SD: 1.9 cm). Nine infants were born with HC<1 SD, 10 with HC<2 SD and 23 with HC<3 SD below the mean for gestational age. Head circumference was also measured on the day of the MRI exam, where 1 infant presented with a normal head circumference, 3 with HC<2 SD below the mean for gestational age, 26 with HC<3 SD below the mean for gestational age and 12 without the measure of head circumference. Timing of maternal infection was self-reported, according to the trimester of pregnancy that mothers had Zika virus symptoms. Twenty-one mothers reported that the infection occurred in the first trimester of pregnancy, 12 in the second trimester, 5 in the third trimester and 4 mothers could not recall the trimester of infection.

Zika virus–microcephaly severity classification

Brain malformations varied considerably across participants (Online Supplementary Material 5 provides brain images for all infants). The observed variation corroborates previous studies about Zika-virus-related microcephaly malformations [19, 20]. Seven infants were classified in the mild range (16.7%), 13 in the moderate range (31%) and 22 in the severe range (52.4%). We obtained an alpha of Cronbach = 0.937, which means that the severity classification items had high consistency. Table 1 presents the imaging characteristics evaluated, their classifications and the intraclass correlation coefficient. Online Supplementary Material 6 shows examples of the mild, moderate and severe categories of each imaging characteristic.

Radiologic interpretation

Results showed a correlation between cephalic perimeter reduction and all imaging characteristics that make up the

Table 1 Imaging characteristics evaluated for the Zika virus–microcephaly severity score

Brain image characteristic	Score					ICC
	0[a]	1	2	3	4	
Cephalic perimeter reduction		Mild	Moderate	Severe	–	0.977
Volume reduction		Mild	Moderate	Severe	–	0.970
Enlarged supratentorial subarachnoid space		Mild	Moderate	Severe	–	0.968
Ventriculomegaly		Mild	Moderate	Severe	–	1
White matter volume reduction		Mild	Moderate	Severe	–	1
Myelination (hypomyelination/demyelination)		Mild	Moderate	Severe	–	1
Gyral pattern simplification (*n* of lobes)		Focal (1)	Moderate (2)	Diffuse (3+)	–	1
Hippocampus		Malrotation	Volume reduction	Malrotation + volume reduction	–	1
Corpus callosum hypoplasia/dysgenesis		Mild	Moderate	Severe	–	0.991
Brain calcifications (*n* of regions)[b]		1	2	3	4+	0.967
Brainstem hypoplasia		Mild	Moderate	Severe	–	0.899
Cerebellar volume hypoplasia		Mild	Moderate	Severe/agenesis	–	0.987
Malformations of cortical development: polymicrogyria/focal pachygyria (*n* of lobes)		Mild (1)	Moderate (2)	Diffuse (3 or +)	–	0.993

-- score does not apply, *ICC* intraclass correlation coefficient between two radiologists for each image interpretation score

[a] 0 = normal

[b] Number of regions: the location of brain calcification evaluated was cortico-subcortical white matter junction, periventricular region, basal ganglia and posterior fossa

Zika virus–microcephaly severity score; the association between cephalic perimeter reduction and the imaging indices suggests that Zika virus has a generalized effect on the brain. Figure 1 shows sagittal MR images for six infants; the images are rank-ordered from lowest to highest Zika virus–microcephaly severity score to illustrate the brain abnormalities characteristic of each severity group.

The correlation between all imaging characteristics is presented in Fig. 2. All correlations were statistically significant ($r \geq 0.32$, $P<0.05$). Individual Zika virus–microcephaly severity scores per participant are presented in Online Supplementary Material 7. We also calculated the correlations between trimester of Zika virus infection and brain abnormalities that make up the Zika virus–microcephaly severity score. Results indicated that trimester of infection was significantly correlated with cephalic perimeter reduction ($r=-0.334$, $P=0.04$), cortical development ($r=-0.655$, $P<0.001$), gyral simplification ($r=-0.654$, $P<0.001$) and calcification ($r=-0.347$, $P=0.033$).

Neuropsychomotor development and brain imaging indices

The neuropsychomotor developmental Bayley-III scores were all below average for infant age. Our results showed a significant correlation between the Zika virus–microcephaly severity score and the Bayley-III scales domains. Bayley-III scores correlated only with head circumference at birth, in all domains: cognitive ($r=0.373$; $P=0.018$), receptive ($r=0.369$; $P=0.019$) and expressive ($r=0.333$; $P=0.036$) communication; and fine ($r=0.46$; $P=0.003$) and gross ($r=0.423$; $P=0.007$) motor skills. There was no significant correlation between Bayley-III scores and head circumference scores on the day of the MRI exam, or Bayley-III scores and head circumference growth ratio. Neither the trimester of infection nor the head circumference growth ratio at the time of developmental evaluation were significantly associated with cognitive, language or motor scores. Correlation results are presented in the Online Supplementary Material 8.

Results showed an association between the imaging characteristics that composed the Zika virus–microcephaly severity scores and the neuropsychomotor developmental outcomes. The association involved fine and gross motor skills, receptive and expressive communication, and cognitive evaluations. On further analysis, increased severity in the posterior fossa (including reduction of cerebellar volume and brainstem) had a correlation with motor skills. Previous studies have shown that alterations in the posterior fossa can cause devastating balance and motor problems [21]. The correlation results are presented in Table 2.

Semi-automated version of the Zika virus–microcephaly severity score

We created a semi-automated method to simplify the generation of the Zika virus–microcephaly severity score, using

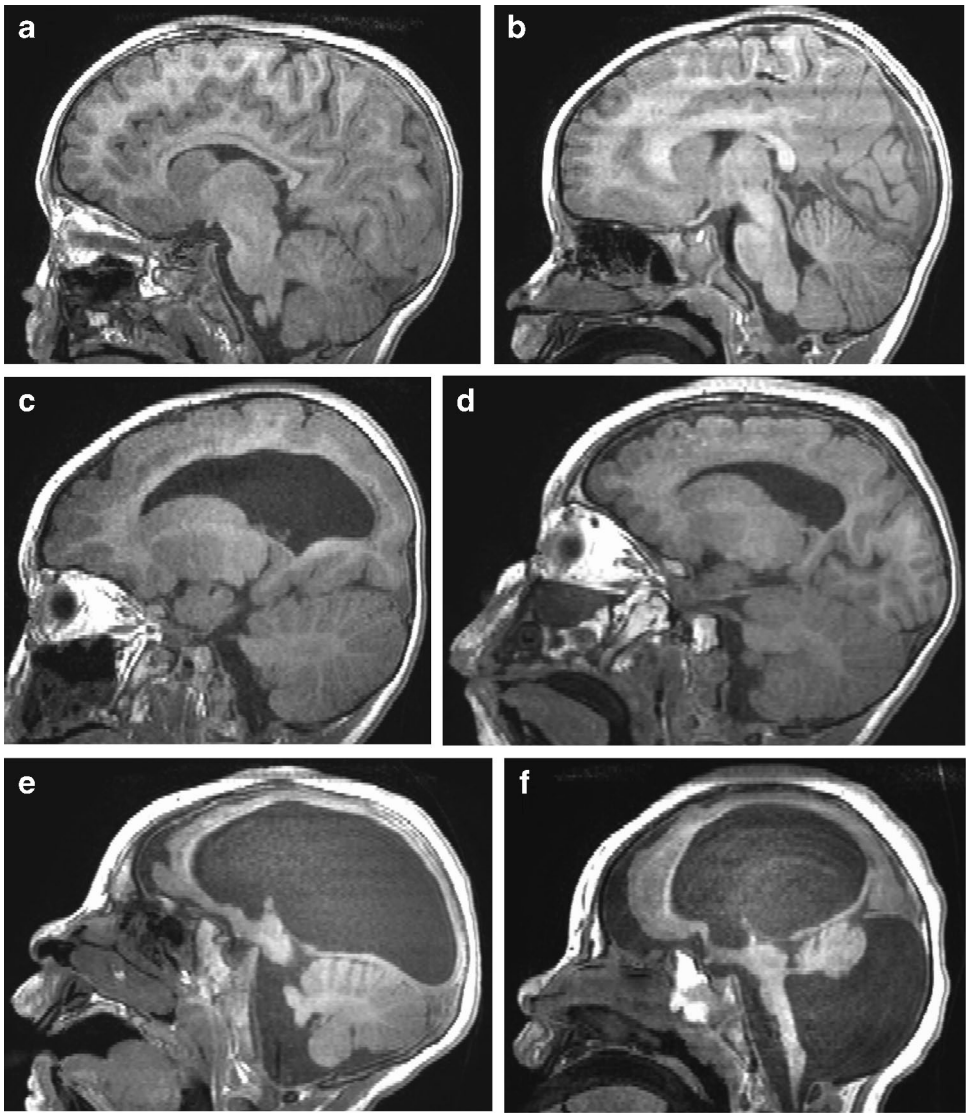

Fig. 1 Sagittal T1-W MR brain images are rank-ordered from lowest to highest Zika virus–microcephaly severity score to illustrate the differences in brain morphology among infants in the three severity groups. **a, b** In the mild group: (**a**) a 29-month-old boy, head circumference at birth (HCb)=32 cm (<1 standard deviation [SD] below the mean), head circumference at MRI exam (HC)=46 cm (<2 SD), Zika virus–microcephaly severity score = 5, and semi-automated score = 3; and (**b**) a 22-month-old girl with HCb=32 cm (<1 SD), HC=41 cm (<3 SD), Zika virus–microcephaly severity score = 6, and semi-automated score = 6. **c, d** In the moderate group: (**c**) a 22-month-old boy, HCb=31 cm (<2 SD), HC=38 cm (<3 SD), Zika virus–microcephaly severity score = 17, and semi-automated score = 17; and (**d**) a 29-month-old boy, HCb=31 cm (<2 SD), HC=42 cm (<3 SD), Zika virus–microcephaly severity score = 22, and semi-automated score = 18. **e, f** In the severe group: (**e**) a 30-month-old girl, HCb=27 cm (<3 SD), HC=40 cm (<3 SD), Zika virus–microcephaly severity score = 33, and semi-automated score = 32; and (**f**) a 27-month-old girl, HCb=25 cm (<3 SD), HC=39 cm (<3 SD), Zika virus–microcephaly severity score = 38, and semi-automated score = 36. Head circumference was expressed in centimeters and normalized by z-score

brain-based volumes and one brain image sequence only (T1-weighted volumetric image). We used a linear regression to predict the severity score (dependent variable) with the measured brain volumes as the independent variable. The β values obtained through linear regression are the following: β_0=51.40, β_1=−12.67, β_2=−4.83, β_3=−10.79, β_4=1.28, β_5=1.31 and β_6=−28.33. The scatter plot of the linear regression is shown in Fig. 3.

We also used the semi-automated score to establish the proposed classification of severity into groups. Using terciles, the scores for the mild group ranged from 0 to 13 and for the moderate group, 14 to 27. Infants with the predicted severity score above 27 were classified into the severe group. Considering the predicted scores, 9 infants were classified in the mild group (21.4%), 16 in the moderate group (38.1%) and 17 in the severe group (40.5%).

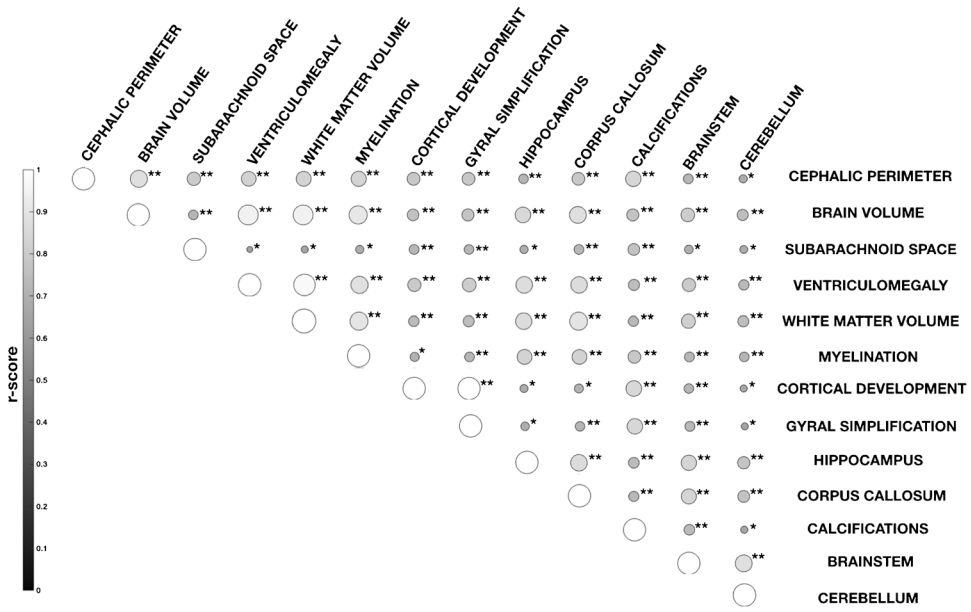

Fig. 2 Correlation matrix among the 13 imaging characteristics evaluated on all 42 infants. Lighter shades of gray and larger circles represent higher correlation (r-score). *P<0.01 and **P<0.001

Table 2 Correlation results between Bayley-III scales and the imaging characteristics of Zika virus–microcephaly severity score

	Cognition r (P-value)	Receptive communication r (P-value)	Expressive communication r (P-value)	Fine motor skills r (P-value)	Gross motor skills r (P-value)
Cephalic perimeter	−0.51[a]	−0.44[a]	−0.41[a]	−0.56[b]	−0.45[a]
Brain volume	−0.59[b]	−0.59[b]	−0.55[b]	−0.67[b]	−0.61[a]
Subarachnoid space	−0.31[a]	−0.43[a]	−0.39[a]	−0.38[a]	−0.41[a]
Ventriculomegaly	−0.56[b]	−0.56[b]	−0.51[a]	−0.64[b]	−0.56[b]
White matter volume	−0.63[b]	−0.62[b]	−0.5[a]	−0.68[b]	−0.58[b]
Myelination	−0.53[b]	−0.45[a]	−0.36[a]	−0.51[a]	−0.31
Gyral pattern simplification	−0.37[a]	−0.38[a]	−0.41[a]	−0.46[a]	−0.37[a]
Hippocampus	−0.38[a]	−0.39[a]	−0.29	−0.45[a]	−0.39[a]
Corpus callosum	−0.53[b]	−0.54[b]	−0.52[a]	−0.61[b]	−0.6[b]
Brain calcifications	−0.28	−0.19	−0.19	−0.31[a]	−0.24
Brainstem	−0.33[a]	−0.27	−0.28	−0.42[a]	−0.4[a]
Cerebellar volume	−0.3	−0.31[a]	−0.31[a]	−0.34[a]	−0.31[a]
Malformations of cortical development	−0.36[a]	−0.37[a]	−0.41[a]	−0.45[a]	−0.37[a]

Two-tailed partial correlation, controlling by age (in months)

[a] P<0.05

[b] P<0.001

Grouping for each infant is provided in Online Supplementary Material 9.

We validated the semi-automated score by performing a bootstrap analysis (1,000 bootstrap samples) in a 75% random sample (32 infants). Here, the β values obtained through linear regression in the random sample were: β_0=54.77, β_1=−1.84, β_2=−5.24, β_3=−27.87, β_4=−9.67, β_5=1.45 and β_6=−14.33. Table 3 shows the correlation results among the 75% random sample, trimester of infection, head circumference and the neuropsychomotor development assessed through Bayley-III.

Comparisons between severity scores and development

Table 4 presents a comparison between the two versions of the severity score: the Zika virus–microcephaly severity score based on 13 image characteristics, and the

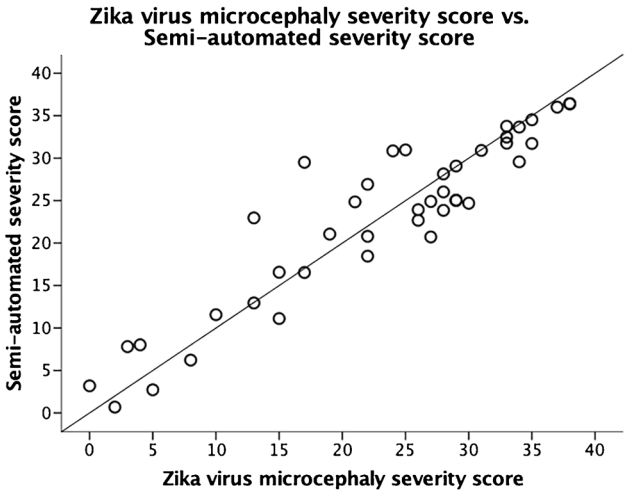

Fig. 3 Zika virus–microcephaly severity score (x-axis) versus semi-automated severity score (y-axis). The dashed line represents y=x. This linear behavior indicates a strong correlation between the two scores (r=0.89; $P<0.001$; two-tailed Spearman)

semi-automated severity score. The trimester of infection correlated only with Zika virus–microcephaly severity score; the trimester is an estimate that is error-prone, and possibly not granular and accurate enough to provide information about severity of microcephaly. However, the head circumference at birth correlated with both severity scores. Head circumference measure at the MRI exam and growth ratio did not significantly correlate with any of the severity scores.

Regarding the neurodevelopment, the severity scores significantly correlated with the neuropsychomotor development assessed through the Bayley-III scales. The correlation was greater for the semi-automated than for the radiologic-based scores. The stronger correlations suggest the semi-automated score provides an assessment of severity that might be informative of the clinical stage of neuropsychomotor development.

Discussion

To our knowledge, this is the first study to propose a brain malformation severity classification for Zika-virus-related microcephaly based on radiologic findings. The severity of microcephaly was associated with poorer developmental scores in all cognitive domains. Head circumference is a widely used parameter for assessing severity of microcephaly [20]. In our study, infants presented with significant variation in brain morphological malformations regardless of head circumference measured at the time of the brain scan (or the head circumference growth ratio). Only head circumference measured at birth showed association with developmental scores. The Zika virus–microcephaly severity score is a more fine-grained evaluation and significantly correlated with developmental scores. This scoring system might capture the effects of Zika virus on brain development with a granularity that allows for investigating prognoses of cognitive development in longitudinal studies of microcephaly. We also showed that brain volume can suffice to classify infants into severity groups (mild, moderate and severe).

Grouping brain malformations according to severity

Grouping infants according to severity (mild, moderate and severe) was based on the more granular categorization of the Zika virus–microcephaly severity score. Thus far, this scoring system seems to better inform the probability of Zika-virus-related microcephaly impacting cognitive and behavior outcomes: it correlated with all Bayley-III scores. However, the semi-automated score has the potential of being further developed and adjusted by establishing, for example,

Table 3 Correlation results among the 75% random sample and neuropsychomotor development

	r	*P*	Standard error	95% CI
Zika virus–microcephaly severity score	0.878	<0.001	0.06	0.716; 0.950
Semi-automated severity score	0.984	<0.001	0.01	0.930; 0.998
Head circumference at birth	−0.739	<0.001	0.08	−0.864; −0.529
Head circumference at MRI exam	−0.380	0.038	0.18	−0.686; −0.02
Head circumference growth ratio	0.187	0.324	0.20	−0.234; 0.537
Trimester of infection	−0.086	0.607	0.16	−0.392; 0.233
Cognition[a]	−0.619	<0.001	0.14	−0.777; −0.219
Receptive communication[a]	−0.595	<0.001	0.14	−0.755; −0.215
Expressive communication[a]	−0.542	<0.001	0.16	−0.748; −0.107
Fine motor skills[a]	−0.713	<0.001	0.11	−0.843; −0.398
Gross motor skills[a]	−0.622	<0.001	0.15	−0.795; −0.190

CI confidence interval

[a] Partial correlation, controlling by age; assessed through Bayley-III scales

Table 4 Comparison between the Zika virus–microcephaly severity score and the semi-automated severity score

	Zika virus–microcephaly severity score	Semi-automated severity score
Score ranges		
Mild	0–12	0–12
Moderate	13–25	13–25
Severe	26–38	26–36
Participants per group, *n* (%)		
Mild	7 (16.7%)	9 (21.4%)
Moderate	13 (31%)	17 (40.5%)
Severe	22 (52.4%)	16 (38.1%)
Correlation analysis with score, r (*P*-value)		
Trimester of infection	−0.37[a]	−0.1 (*P*=0.5)
Head circumference at birth	0.65[b]	−0.67[b]
Head circumference at MRI exam	−0.35 (*P*=0.054)	−0.3 (*P*=0.08)
Head circumference growth ratio	0.15 (*P*=0.4)	0.2 (*P*=0.1)
Cognition[c]	−0.58[b]	−0.6[b]
Receptive communication[c]	−0.56[b]	−0.59[b]
Expressive communication[c]	−0.52[b]	−0.54[b]
Fine motor skills[c]	−0.66[b]	−0.7[b]
Gross motor skills[c]	−0.57[b]	−0.62[b]

[a] $P<0.05$

[b] $P<0.001$

[c] From the Bayley-III assessment; two-tailed Spearman correlation. Partial correlation used for correlations with Bayley-III scales, controlling for age

a weighted score for abnormalities and indices that have a more significant load on development. The grouping of malformations according to the severity score could be tested in future evaluations against neurodevelopmental outcomes in infants with congenital microcephaly.

Semi-automated Zika virus–microcephaly severity score

We have shown that there is a significant correlation between the Zika virus–microcephaly severity score and the semi-automated scores based on the VOIs (r=0.891; $P<0.001$). However, there were disagreements between the clinical and the semi-automated scores. Infant ID = Z101 had a Zika virus–microcephaly severity score of 17 and a semi-automated score of 30. These scores categorize microcephaly as moderate according to the radiologic findings, but as severe according to the semi-automated method. Visual inspection of the images suggests the semi-automated score is biased by enlarged ventricles and reduced cerebral cortex volume. Again, further development of the score and testing with other microcephalic populations might help adjust, for example, how indices are weighed in the semi-automated score. Nonetheless, the semi-automated method for measuring brain abnormalities can inform neuroradiologic findings and neurologic evaluations. The training required to perform the segmentations requires moderate knowledge about brain anatomy and the use of a software called "Insight Segmentation and Registration Toolkit" (ITK-SNAP; University of Pennsylvania and University of Utah) [18]. It is expected that with training in the procedures, the results could be easily replicated across research studies.

Study limitations

The study limitations include small sample size relative to the number of microcephalic infants born during the Zika epidemic. Larger and more heterogeneous samples would allow for a better evaluation of replicability and generalizability of the proposed system and its ability to inform prognoses. We underscore that sample size and heterogeneity were limited by the timing of the outbreak relative to the beginning of the study (the outbreak was mostly contained once we started investigating the effects, which limited the ability to include newborns and to observe pregnancies) and by logistical challenges (participants were flown from northeastern regions of the country, more than 3,600 km away from our research site, to participate in the study). Additionally, the study did not have a control group and the method was not applied to either controls or another population of infants with brain abnormalities. Last, Zika virus congenital infection can result in neuronal loss, disruption and

destruction; however, our study was limited to the assessment of malformations. Anatomopathological data were not collected in the present study.

Conclusion

This study provides a method for severity classification of brain abnormalities, establishing three categories: mild, moderate and severe microcephaly. This severity classification relies on assessment of a combination of alterations in brain structures and indices; is thus more granular and possibly more promising for understanding patient prognoses relative to classification of severity based on head circumference alone. The classification system must be further tested and evaluated. The proposed classification might apply to other populations with microcephaly (not related to Zika virus) or with other congenital, brain-related diseases.

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Declarations

Conflicts of interest None

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