Brain Malformations in Zika Virus Related Congenital Microcephaly: interactions among clinical and imaging scores and a semi-automated classification of severity based on MRI indices (RaRe - Radiological Reading score)

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ABSTRACT

Objectives: We propose and evaluate a system for assessing severity of ZikV-related microcephaly brain abnormalities called RaRe (Radiological Reading score). The system is a combination of neuroradiologist evaluation scores; we also propose and evaluate its semi-automated version, which is generated from MRI indices.

Design: We carried out a cross-sectional study with retrospective data collection at birth and 13-39 months; data collection includes radiologists evaluation of MRI images and a semi-automated system for analyzing MRI images from ZikV-related microcephaly patients; we also evaluated the association of severity and RaRe scores with developmental outcomes.

Setting: Brain Institute of Rio Grande do Sul and Memorial Hospital Arthur Ramos, both in Brazil.

Participants: 42 infants with ZikV-related congenital microcephaly (age at second evaluation M = 24.83 months; SD = 5.82). Mothers were infected by Zika Virus during pregnancy and were symptomatic or asymptomatic.

Main outcome measures: Clinical and radiological outcomes (blindly reviewed); severity classification system (semi-automated – RaRe-Auto or based on neuroradiological evaluation – RaRe-Clin) and association with developmental outcomes (Bayley-III scales).

Results: RaRe-Clin positively correlated with RaRe-Auto (r = 0.892; p < 0.001). Severity negatively associated with neurodevelopment scores assessed by Bayley-III scales in all three domains: cognition, language and motor skills. There was no association between trimester of infection and head circumference at birth (r = 0.160; p = 0.338) but there was an association between trimester of infection and RaRe-Clin (r = -0.355; p = 0.029).

Conclusions: The clinical and semi-automated RaRe scores predicted Bayley scores. RaRe-Clin and RaRe-Auto can predict the trimester of infection and severity of outcomes and should be investigated in their further application with other congenital and infection-related brain abnormalities.
What is already known on this topic:

- Congenital ZikV infection is associated with increased risk for microcephaly;
- Microcephaly is associated with severe cognitive deficits and brain abnormalities;
- Head circumference at birth < 32 cm establishes the diagnosis of microcephaly but other indices are needed to glean information about severity of abnormalities and prognoses

What this study adds

- We propose a composite scoring system (RaRe) and system for grouping patients according to severity of microcephaly based on clinical indices (radiological readings of microcephaly; RaRe-Clin) and semi-automated MRI-based scores (RaRe-Auto).
- The proposed scoring systems are associated with developmental outcomes associated with ZikV-related microcephaly and trimester of infection and may help inform the prognosis of ZikaV-related microcephaly;
INTRODUCTION

Outbreaks of infectious diseases that affect fetal and newborn brain development present a challenge to healthcare and healthcare-related fields involved in generating clinically and scientifically-relevant evidence for diagnoses and prognoses. These fields include neuroradiology and developmental brain imaging, a combination that can allow for the translation of brain imaging indices to clinical practice. In 2015, there was an epidemic of Zika Virus infection (ZikV) in the Northeast of Brazil. Newborns of mothers infected with ZikV during pregnancy presented severe lesions in the central nervous system (1,2) and abnormal brain development and malformation, most notably, microcephaly (3,4). Between March 2015 and February 2016, there were over 4,000 births of children with microcephaly (head circumferences < 32 cm below average for sex and gestational age). These 4,000 births of microcephalic babies represented a 20-fold increase in microcephaly in Brazil for an equivalent time period (5,6).

The identification of ZikV RNA in the amniotic fluid of mothers whose fetuses had cerebral abnormalities was central to the association among infection and risk for abnormal brain development. It suggested ZikV transmission occurred during pregnancy (7–11). ZikV infection has been found in microcephalic fetus brains (12) and is associated with increased neural cell death in infected stem cells (13); it has been shown to target human brain cells and reduce growth and viability (14). ZikV infection between the first and second trimester of pregnancy represents higher risk for microcephaly relative to infection in the third trimester (15,16). Primary congenital microcephaly is associated with abnormal brain development (17,18). Head circumference establishes the diagnosis of microcephaly but, of course, does not provide information to establish a prognosis for brain development, which include learning and intellectual disabilities and lifelong neurological impairments (19). The central nervous system (CNS) lesions include destructive, calcification, hypoplasia and migration disturbances of the CNS (20); moreover, studies of developmental outcomes show that severity of microcephaly was associated with severity of cognitive (21) and motor (22) impairments. In an endemic area of Brazil, cerebral calcifications were identified as a predominant outcome in ZikV-related microcephaly. The calcifications affected periventricular regions, the cerebral parenchyma, thalamus, and basal ganglia; neuronal migration abnormalities were also reported (23). Obstetric ultrasound findings include gross cerebral calcifications, cerebellar vermis abnormalities and corpus callosum dysgenesis (9). The brain is thus susceptible to the effects...
of ZikV infection in multiple developmental stages (24,25). For patients of mild microcephaly not caused by ZikV, behavioral and clinical outcomes have been show to vary: about 50% of cases of mild microcephaly not caused by ZikV have normal intelligence scores (26). The prognoses of ZikV-related microcephaly are one of mild to severe developmental impairments; the challenge addressed by the present study was to establish a combination of clinical and brain imaging indices that can help inform clinical prognoses.

The goal of the present study was to propose and evaluate two composite scores for diagnosis of severity of ZikV-related microcephaly: one that is based on neuroradiological readings of MRI images (named RaRe-Clin) and another based on semi-automated scoring of MRI images (RaRe-Auto); we named the general scoring system RaRe (Radiological Readings). To our knowledge, there are no brain imaging studies of ZikV-related microcephaly that approached the relationship between microcephalic neural indices associated with ZikV infection severity and neuroradiological reading scores. We investigated such relationship and proposed, for the first time, a classification of severity of ZikV-related microcephaly using (human-based) scores of MRI readings and propose a semi-automated method for obtaining these MRI indices.

**METHODS**

*Participants*

The study included 42 congenital microcephalic infants (19 females, mean age = 24.83 months, SD = 5.82 months) registered at a state Health Department with suspected or confirmed congenital ZikV infection. Inclusion criteria for mothers and infants followed Brazilian Ministry of Health guidelines (19). The criteria for mothers were history of pruritic maculopapular exanthema and positive ZikV Immunoglobulin M (IgM) serological reaction; or clinical-epidemiological measures and at least two of the following symptoms: fever, pruritus, arthralgia of multiple joints and/or periarticular edema. Inclusion criteria for infants were positive for ZikV Immunoglobulin G (IgG) and born to mothers with suspected or confirmed ZikV infection during pregnancy. Mothers and infants were screened for the following congenital infections disorders: syphilis, toxoplasmosis, rubella, cytomegalovirus, herpes simplex - STORCH. No infant participant tested positive for any of these infections. Written informed consent was obtained 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 (CAEE 61642016.6.1001.5336).

Mean birth weight was 2650g (SD = 0.530g), 37 were term and mean head circumference (HC) at birth was 29.81 cm (SD = 1.89 cm). Nine infants were born with HC < 1SD, ten with HC < 2SD and twenty-three with HC < 3SD below the mean for gestational age. Head circumference was also measured on the day of the MRI exam, where one infant presented a normal HC, three with HC < 2SD, twenty-six with HC < 3SD and twelve without the measure of HC. Out of total participants, twenty-one reported that the infection occurred in the first trimester, twelve in the second trimester, five in the third trimester and four could not recall the trimester of infection.

*Instruments and Procedures*

Bayley Scales of Infant and Toddler Development (Bayley-III) scores were collected one day after the MRI exam. We used the translated Brazilian Portuguese of Bayley-III (27) to assess three domains: cognition, language (receptive and expressive communication) and motor (gross and fine). HC was measured at birth and at the MRI exam; we thus have two HC measures and we generated a third HC score, which we called HC growth ratio. The HC growth ratio is the difference between HC on the day of the MRI exam and HC at birth divided by HC at birth.

*MRI Data acquisition*

Twenty-eight infants were scanned at 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. Brain Institute: The data was collected using a GE HDxT 3T MRI scanner with an 8-channel head coil. T1 structural scans were acquired with the following parameters using a BRAVO sequence: repetition time (TR) = 6.16ms, echo time (TE) = 2.18ms, flip angle = 8°, acquisition matrix of 240 x 240 x 196 and voxel size of 1.0 x 1.0 x 1.0mm. Memorial Hospital: The data was collected on an Optima MR450W 1.5T MRI scanner with a 16-channel head coil. The T1 structural scans were acquired with the following parameters using a BRAVO sequence: TR = 8.7ms, TE = 3.224ms, flip angle = 12°, acquisition matrix = 256 x 256 x 100 and voxel size = 0.938 x 0.938 x 1.2mm. To avoid motion artifacts in the images, participants
in both sites were anesthetized and the MRI exams were carried out under the supervision of an expert anesthesiologist.

**Clinical Radiological Reading score (RaRe-Clin)**

Postnatal brain MRI images were analyzed by two neuroradiologists (R.B.S and R.C.B). They performed independent analysis of the MRI images and were blind to patient clinical history and to the evaluations of one another. After evaluations were performed, the disagreements in evaluations were resolved by means of reaching a consensus in a case-by-case discussion. Intraclass Correlation Coefficient (ICC) was calculated among each one of the 14 characteristics; it showed an excellent score (>0.8). ICC is presented in Table 1. Brain MRI images were reviewed to investigate structural abnormalities based on the following 14 characteristics: reduced cephalic perimeter, reduced brain volume, enlarged anterior supratentorial subarachnoid space, cerebral ventricular enlargement, decreased white matter volume, evaluation of myelination (hypomyelination or demyelination), gyral pattern simplification, hippocampus (normal, volumetric reduction, malrotation, volumetric reduction and malrotation), abnormalities of the corpus callosum (classified as mild/moderate/severe hypoplasia or dysgenesis), presence and location of brain calcifications, brainstem hypoplasia, decreased cerebellar volume, cystic malformation of posterior fossa and malformations of cortical development. Except for calcification, the characteristics were scored on a 4-point scale from zero to 3, being zero normal and 3 the most severe abnormality according to the neuroradiologist. The brain calcifications were scored on a 5-point scale from zero to 4, being zero normal and 4 presenting calcifications in 4 or more regions. The main brain regions analyzed for the calcifications were cortico-subcortical white matter junction, periventricular, basal ganglia and posterior fossa. There were disagreements between two neuroradiologists in 24 of 588 characteristics. We thus propose a score based on the Clinical Radiological Reading (RaRe-Clin) that is the sum of the 14 items. The maximum RaRe-Clin score is 43. Table 1 presents the characteristics evaluated and their classification.
Table 1 – Description of the image interpretation for the Clinical Radiological Reading score (RaRe-Clin).

| Brain Image Characteristic                      | 0 * | 1   | 2   | 3   | 4   | ICC |
|------------------------------------------------|-----|-----|-----|-----|-----|-----|
| Perimeter Reduction                            |     | Mild| Moderate | Severe | N/A | 0.977 |
| Volume                                         |     | Mild| Moderate | Severe | N/A | 0.970 |
| Supratentorial Subarachnoid Space              |     | Mild| Moderate | Severe | N/A | 0.968 |
| Ventriculomegaly                               |     | Mild| Moderate | Severe | N/A | 1    |
| White Matter Volume Reduction                  |     | Mild| Moderate | Severe | N/A | 1    |
| Myelination (hypomyelination/demyelination)    |     | Mild| Moderate | Severe | N/A | 1    |
| Gyral Pattern Simplification (n of lobes)      |     | Focal (1) | Moderate (2) | Diffuse (3+) | N/A | 1    |
| Hippocampus**                                  |     | Malrot. | Vol. reduc. | Malrot. + vol. reduc. | N/A | 1    |
| Corpus Callosum hypoplasia/ dysgenesis         |     | Mild| Moderate | Severe | N/A | 0.991 |
| Brain Calcifications (n of lobes)***           |     | 1   | 2   | 3   | 4+  | 0.967 |
| Brainstem hypoplasia                           |     | Mild| Moderate | Severe | N/A | 0.899 |
| Cerebellar Volume hypoplasia                   |     | Mild| Moderate | Severe/agenesis | N/A | 0.987 |
| Cystic Malformation of Posterior Fossa         |     | MMC | DWV | DW  | N/A | 0.988 |
| Malformations of Cortical Development:         |     | Mild (1) | Moderate (2) | Diffuse (3 or +) | N/A | 0.993 |
| polymicrogyria/focal pachygyria (n of lobes)   |     |     |     |     |     |     |

*0 = Normal. ** Malrot. = Maltortion; Vol. Reduc. = Volume Reduction. *** Number of regions: The location of brain calcification evaluated was cortexo-subcortical white matter junction, periventricular, basal ganglia and posterior fossa. MMC = Magna Mega Cistern. DWV = Dandy Walker Variant. DW = Dandy Walker. ICC = Intraclass Correlation Coefficient between two radiologists for each image interpretation score. N/A = the score of 4 does not apply.

Prediction of Severity of Microcephaly using Semi-Automated RaRe (RaRe-Auto)

Neuroradiological readings can be laborious and they depend on experienced medical doctors. We present a method to semi-automatically obtain the RaRe score and thus establish severity of microcephaly-related abnormalities based on volumetric measures. Volumetric measures were obtained using a standardized tracing protocol (28,29) that measured four volumes of interest (VOI), for each participant. The volumes were: (a) the lateral ventricles, (b) whole brain, (c) intracranial, and (d) the cerebellum segmentation (Supplementary Figure 1). We applied a semi-automated region growing segmentation method based on the edge detection algorithm in ITK-SNAP (30). The procedure for segmentation of the VOIs is described in the Supplementary Materials.

The semi-automated method RaRe-Clin score allows for replication of evaluation across sites investigating possible ZikV outbreaks, or other infections that cause congenital brain abnormalities. The regression model and the parameters are described in the Supplementary Material.
Statistical Analysis
We carried out statistical analyses using the Statistical Package for Social Sciences (SPSS), version 23. We used descriptive statistics for the population demographics. We used Kolmogorov-Smirnov (KS) 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 KS test showed that all variables had a non-parametric distribution. Next, we applied Spearman correlation to investigate the relationship among variables. We used partial correlations and controlled for age for the analyses of Bayley Scales and the clinical and image variables. A p < 0.05 was considered statistically significant for all analyses.

Patient and public involvement
This research was done without patient, parents or guardians’ involvement. Patients, parents or guardians were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients, parents or guardians were not invited to contribute to the writing or editing of this document for readability or accuracy.

RESULTS
ZikV Brain Malformation Classification
Brain malformations varied considerably across participants (Supplementary Figure 2) and this variation corroborates previous studies about ZikV-related microcephaly malformations (31–33). We propose a scoring system called RaRe-Clin for brain abnormalities that is based on clinical evaluations and that make up the proposed classification system. The system is based on a score of 14 malformation characteristics of the MRI structural scans, which we propose may be used as a ZikV Brain Malformation Severity (ZikV-BMS) index. The ZikV-BMS allows for classification of severity of malformations into three groups: Mild, Moderate and Severe. The RaRe-Clin for the Mild group ranged from 0 to 13 and for the Moderate group 14 to 27. Participants with RaRe-Clin above 28 where classified into the Severe group. A zero RaRe-Clin score indicates that the classification method identified minimal brain malformation. Using the proposed classification system, the trimester of maternal infection significantly correlated with the ZikV-BMS grouping (r = -0.473, p = 0.003). Supplementary
Table 1 shows in which ZikV-BMS group each participant was classified into. The inter-rater reliability for RaRe-Clin was excellent (ICC = 0.9817), as described in Table 1.

**Predicting RaRe using volumetric information**

Results showed a correlation of $r = 0.892$ ($p < 0.001$) between the RaRe-Clin score, given by two neuroradiologists, and the RaRe-Auto score, based only on volumetric measurements. We created a method to predict the RaRe-Clin by using the semi-automated brain-based volumes. Specifically, we used a linear regression to predict the RaRe-Clin (dependent variable) by using the measured brain volumes as independent variables. The scatter plot of the linear regression prediction of RaRe-Auto is shown in Figure 1. This result indicates that the use of a semi-automatic volumetric measurement can be used to reliably predict the RaRe-Clin.

![Figure 1](image)

**Figure 1** – RaRe-Clin (x axis) versus RaRe-Auto score (y axis). The dashed line represents $y = x$. This linear behavior indicates a strong correlation between the RaRe-Clin and the RaRe-Auto score ($r = 0.892; p < 0.001; 2$-tailed; Spearman).

*Clinical and Imaging Interactions*
There was no significant correlation between head circumference measured at birth and trimester of infection ($r = 0.160, p = 0.338$). The results show a significant association between RaRe-Clin with the trimester of suspected maternal infection ($r = -0.355; p = 0.029$). No other clinical variable presented a significant correlation with the trimester of infection. The RaRe-Clin score showed a significant negative correlation with brain volume, suggesting they are inversely proportional ($r = -0.846, p < 0.001$). Figure 2 shows the correlations between the clinical information (trimester of infection, and head circumference at birth and on day of MRI exam) and brain imaging measurements. The correlation between growth ratio and trimester of infection was not statistically significant ($r = 0.015, p = 0.94$).
| TRIMESTER OF INFECTION | HC AT BIRTH | HC AT MRI EXAM | RARE-CLIN SCORE | INTRACRANIAL VOLUME | BRAIN VOLUME | VENTRICLES/INTRACRANIAL VOLUME |
|------------------------|-------------|----------------|-----------------|--------------------|--------------|--------------------------------|
|                        | ![Bar Chart](image1) | ![Bar Chart](image2) | ![Bar Chart](image3) | ![Bar Chart](image4) | ![Bar Chart](image5) | ![Bar Chart](image6) |
| HC AT BIRTH             | r = 0.160   | p = 0.338      | ![Correlation](image7) | ![Correlation](image8) | ![Correlation](image9) | ![Correlation](image10) |
| HC AT MRI EXAM          | r = 0.124   | p = 0.524      | ![Correlation](image11) | ![Correlation](image12) | ![Correlation](image13) | ![Correlation](image14) |
| RARE-CLIN SCORE         | r = -0.355  | p = 0.029      | ![Correlation](image15) | ![Correlation](image16) | ![Correlation](image17) | ![Correlation](image18) |
| INTRACRANIAL VOLUME     | r = 0.090   | p = 0.589      | ![Correlation](image19) | ![Correlation](image20) | ![Correlation](image21) | ![Correlation](image22) |
| BRAIN VOLUME            | r = 0.113   | p = 0.5        | ![Correlation](image23) | ![Correlation](image24) | ![Correlation](image25) | ![Correlation](image26) |
| VENTRICLES/INTRACRANIAL VOLUME | r = -0.076  | p = 0.648      | ![Correlation](image27) | ![Correlation](image28) | ![Correlation](image29) | ![Correlation](image30) |

Figure 2 – Correlation of the trimester of infection, head circumference at birth and on day of MRI exam, and brain imaging measurements. The main diagonal shows the histogram with nonparametric kernel-smoothing distribution. The top right triangle shows the correlation between the variables. Spearman correlation and p-value are presented in the lower left triangle. HC = head circumference; MRI = magnetic resonance imaging; RaRe-Clin = Clinical Radiological Reading score. Ventricular volume was normalized across participants by dividing by the intracranial volume.
Neuropsychological and Imaging Interactions

Infants presented neurodevelopmental delays in all Bayley-III scores (below average for age). We used the raw score to perform analysis of correlations among Bayley-III cognitive, receptive and expressive communication, and fine and gross motor scores with RaRe. Statistical results showed a significant correlation between the RaRe-Clin score and all three domains of Bayley-III scales: cognitive (r = -0.580, p < 0.001), receptive communication (r = -0.565, p< 0.001), expressive communication (r = -0.531, p < 0.001), fine motor (r = -0.664, p < 0.001) and gross motor (r = -0.577, p < 0.001). Similarly, results showed significant correlation between the RaRe-Auto and the Bayley-III scores for all domains: cognitive (r = -0.593, p < 0.001), receptive communication (r = -0.582, p < 0.001), expressive communication (r = -0.530, p < 0.001), fine motor (r = -0.692, p < 0.001) and gross motor (r = -0.615, p < 0.001). There was no significant correlation among Bayley-III scores and head circumference scores (at the MRI exam and growth ratio). There was a significant correlation among Bayley scores and head circumference at birth for all domains: cognitive (r = 0.373; p = 0.018), receptive communication (r = 0.369; p = 0.019), expressive communication (r = 0.333; p = 0.036), fine motor (r = 0.460; p = 0.003) and gross motor (r = 0.423; 0 = 0.007). A description of the correlation results is shown in Supplementary Table 2.

Radiological Interpretation

Results showed a correlation among cephalic perimeter volume and all image characteristics that make up the RaRe-Clin score (r value range 0.36 (p<0.05) to 0.76 (p<0.001)); the association among cephalic perimeter volume and the imaging indices suggests ZikV has a generalized effect on the brain's gray and white matter development, on myelination, on calcification and all other brain development abnormalities investigated (see Table 1). The correlations among the separate measures are presented in the Supplementary Figure 3. We present sagittal MRI images for nine subjects in Figure 3; the images are rank-ordered from lowest to highest RaRe-Clin scores to illustrate the severity of brain abnormalities at each stage. Sagittal MRI images for all participants are included in Supplementary Figure 2.

Results showed positive correlation between white matter volume and ventricular volume (r = 0.95, p<0.001). This indicates that the reduction of brain volume in these patients is associated with the enlargement of ventricular volume. The results also show an association among the volume of cerebrospinal fluid (CSF) in the outer brain (space between the cortex and the cranial bone) and reduction of white matter volume (r = 0.464, p = 0.002), and the ventricular volume
enlargement ($r = 0.420$, $p = 0.006$). Individual RaRe-Clin per participant are presented in Supplementary Table 4.

The correlations among the trimester of ZikV infection and brain abnormalities that make up the RaRe-Clin score showed that trimester of infection was significantly correlated with cephalic perimeter volume ($r=-0.334$, $p=0.04$), cortical development ($r = -0.655$, $p < 0.001$), gyral simplification ($r = -0.654$, $p < 0.001$), and calcification ($r = -0.347$, $p = 0.033$).

Figure 3 – Sagittal MRI images to illustrate differences in brain morphology among nine participants. SD = standard deviation; HCB = head circumference at birth; HC = head circumference at MRI exam; RaRe-Clin = Clinical Radiological Reading Score; RaRe-Auto = Predicted Radiological Reading Score (using volumetric measures). Head circumference was expressed in centimeters and normalized by Z-Score. Images were rank-ordered from lowest to highest RaRe-Clin scores.
DISCUSSION

To our knowledge, this is the first study to propose a brain malformation severity classification system for ZikV (ZikV-BMS) infected patients based on a score system for radiological readings. The severity of the disease was associated with brain morphological malformations and there was a significant correlation among radiological image characteristics. The results show abnormal brain indices in association with microcephaly. Based on a cumulative score of brain malformation (RaRe-Clin), we propose to characterize the severity of the illness in three categories: mild, moderate and severe. We also presented a semiautomated method (RaRe-Auto) to measure the volume of brain regions, which is independent of the neuroradiological readings, we can predict the RaRe-Clin with high precision.

Brain Malformation Scoring

Head circumference is a widely used parameter for assessing severity of microcephaly (32). Our study shows infants presented significant variation in brain morphological malformations regardless of head circumference and trimester of suspected infection (see Figure 3 and Supplementary Figure 2). The RaRe-Clin, in turn, encompasses more comprehensive information about brain malformation, which significantly correlated with trimester of suspected maternal infection. The proposed RaRe-Clin scoring system may capture the effects of ZikV on brain development with a granularity that allows for establishing prognoses of cognitive development.

Grouping Brain Malformations according to Severity

The grouping of participants according to severity (mild, moderate, and severe) is based on the more granular categorization of the RaRe-Clin score. The RaRe-Clin scoring system may better inform the probability that ZikV-related microcephaly will impact cognitive and behavior outcomes. The grouping of malformations according to the proposed severity scores may be tested in future evaluations against neurodevelopmental outcomes in infants with congenital microcephaly. Head circumference alone informs the presence of microcephaly but does not predict the outcomes of cognitive and brain development.
Prediction of the image score based on volumetric measures

We have shown that there is a significant correlation between the RaRe-Clin and RaRe-Auto score based on the VOIs ($r = 0.892; p < 0.001$). However, there were some cases where there was a large disagreement between both mentors. Participant ID = 101 had a RaRe-Clin score of 17 and RaRe-Auto score of 30. The participant belonged to the moderate group according to the human radiological reading; the RaRe-Auto score puts the participant in the severe group. The same occurred for participant ID = 023, who was classified as moderate by the RaRe-Clin and severe by the RaRe-Auto. Visual inspection of the images suggests the RaRe-Auto score is biased by the enlarged ventricles and reduced cerebral cortex volume. Nonetheless, the semi-automated method for measuring brain abnormalities can inform neuroradiological readings and neurological evaluations. The proposed semi-automated RaRe-Auto requires about 4 hours of manual labor to calculate the VOI per sample. The training required to perform the segmentations requires knowledge about brain anatomy and about the use of the software (ITK-Snap). It is expected that with training in the procedures the results can be replicated.

Relationship among RaRe and Bayley outcomes

The results showed an association among the RaRe (RaRe-Clin and RaRe-Auto) and developmental outcomes measured by Bayley-III scales. The association involved fine and gross motor, receptive and expressive communication, and cognitive evaluations. On further analyses, increased severity in the posterior fossa malformations had a correlation with motor skills (fine motor: $r = -0.389$, $p = 0.013$; gross motor: $r = -0.325$, $p = 0.038$). Previous studies have shown that alterations in the posterior fossa can cause devastating balance and motor problems (34). The correlation table is presented in the Supplementary Table 3.

Study Limitations

The study is limited in the sense that it has a small sample size, considering the number of microcephaly infants born at the outbreak. With a larger sample, a more generalizable severity classification system could be proposed. We have also not investigated other methods to automatically predict the RaRe-Clin, such as using machine learning techniques. However, to use these methods, a much larger sample size is required. The proposed automated method could be tested with other datasets of infants with congenital microcephaly associated with other infections.
Acknowledgments
The authors would like to thank all the participants and their families for cooperate in this study.

Contributors: NBE, AB, ARF, GR, DIC, MWP, MLN and JCC conceived and designed the study. GR did all the logistics to bring mothers and infants to Brain Institute. NBE, KBE, AK and WP did the image segmentation. NBE, AB and ARF did the literature review. RBS and RCB contributed to the interpretation of structural images and with the image score. FK did the neurological evaluation. LSA, NM and DIC did the neuropsychological evaluation. NBE did the statistical analysis. NBE, ARF and AB wrote the manuscript, and all authors critically reviewed and approved the final version. The corresponding author attests that all listed authors meet authorship criteria and that no other meeting the criteria have been omitted. NBE and AB are the guarantors.

Funding: This study was sponsored by a grant from FINEP (PUCRS/FINEP 0261/16). Magda Lahorgue Nunes is supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq Brazil grant PQ 306338/2017-3. Jaderson Costa da Costa is supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq Brazil grant PQ 307372/2015-4. Augusto Buchweitz is supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq Brazil grant PQ 311365/2018-3. Nathalia Bianchini Esper was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Competing interests: Authors have no conflict of interest or disclosures to declare.

Ethical approval: The study was approved by the Research Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (CAEE 61642016.6.1001.5336). Written informed consent was obtained from the parents or guardians of all the infants included in this study.

Data sharing: Technical and statistical code are available from the first author at nathalia.esper91@edu.pucrs.br

Transparency declaration: The corresponding author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects
of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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