Pediatric neurodevelopment by prenatal Zika virus exposure: A cross-sectional study of the Microcephaly Epidemic Research Group Cohort

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Abstract

Background: The implications of congenital Zika Virus (ZIKV) infections for pediatric neurodevelopment and behavior remain inadequately studied. The aim of this study is to investigate patterns of neurodevelopment and behavior in children with different levels of ZIKV-related microcephaly or with prenatal ZIKV exposure in the absence of microcephaly.

Methods: We conducted a cross-sectional study, nested in a cohort, of 274 children (aged 10-45 months) who were born during the peak and decline of the microcephaly epidemic in Northeast Brazil. Participants were evaluated between February 2017 and August 2019 at two tertiary care hospitals in Recife, Brazil. We analyzed the children in four groups assigned based on clinical and laboratory criteria: Group 1 had severe microcephaly; Group 2 had moderate microcephaly; Group 3 had prenatal ZIKV exposure confirmed by maternal RT-PCR testing and no microcephaly; and Group 4 was a neurotypical control group. Groups were evaluated clinically for neurological abnormalities and compared using the Survey of Wellbeing of Young Children (SWYC), a neurodevelopment and behavior screening instrument, and a SWYC adapted form to compare severe cases.

Results: Based on the SWYC screening, we observed differences between the groups for developmental milestones but not behavior. Among children with severe microcephaly of whom 98.2% presented with neurological abnormalities, 99.1% were at risk of development delay, and presented similar performance whether evaluated under or over 24 months of age. Among children with moderate microcephaly of whom 60% presented with neurological abnormalities, 65% were at risk of development delay. For children without microcephaly, the percentages found to be at risk of developmental delays were markedly lower and did not differ by prenatal ZIKV exposure status: Groups 3, 13.8%; Group 4, 21.7%.

Conclusions: Among groups of children with prenatal ZIKV exposure, we found a gradient of risk of development delay. Children with severe microcephaly were at highest risk, while normocephalic ZIKV-exposed children had similar risks to unexposed control children. We propose that ZIKV-exposed children should undergo first-line screening for neurodevelopment and behavior using the SWYC. Early assessment and follow-up will enable at-risk children to be referred to a more comprehensive developmental evaluation and to multidisciplinary care management.

Background

Zika virus (ZIKV) is a teratogenic arthropod-borne flavivirus and its vertical transmission can lead to fetal injury, which can clinically manifest in a pattern of signs and symptoms recognized as Congenital Zika Syndrome (CZS). The CZS phenotype is marked by structural defects, including morphological alterations to the limbs, eyes, and brain, as well as functional impairments, such as difficulty in swallowing and communication.

Current evidence suggests that many of the functional disabilities associated with CZS arise from damage to the developing nervous system. Intrauterine ZIKV infections exhibit a marked neurotropism,
and recent studies indicate that ZIKV is able to impair the viability and growth of neural progenitor cells and post migratory neurons.\textsuperscript{3-5} Indeed, microcephaly - a hallmark of CZS - is thought to arise when prenatal infections with ZIKV trigger fetal brain disruption sequence, resulting in brain volume loss, reduced intracranial pressure, and skull collapse.\textsuperscript{2,6}

Despite an increasing understanding of the pathogenic mechanisms of fetal ZIKV infection and the resultant structural changes, the long-term implications of congenital ZIKV exposure on neurodevelopment and behavior remain understudied.

To address the pressing need for research on prognosis of children exposed to ZIKV during pregnancy, we evaluated children enrolled in the Cohort of Children of the Microcephaly Epidemic Research Group (MERG) using the Survey of Wellbeing of Young Children (SWYC) screening instruments,\textsuperscript{7-9} validated for use in Brazil.\textsuperscript{10} Although there are several instruments able to identify signs of risk for developmental delays,\textsuperscript{11} the SWYC is particularly advantageous due to its ease of use as a first-line screening tool and its capacity to assess children with varying degrees of neurological impairment.

The aim of this study is to investigate the neurodevelopment and behavior of children with ZIKV-related microcephaly or with prenatal ZIKV exposure in the absence of microcephaly born between 2015 and 2017 in Pernambuco State, the epicenter of the microcephaly epidemic in Northeast Brazil.\textsuperscript{12}

**Methods**

We conducted a cross-sectional study, nested in the MERG Pediatric Cohort of children born during the peak and decline of the ZIKV-related microcephaly epidemic in the Northeast of Brazil. Ethical approval for the recruitment, follow-up, and screening of the children was provided by local institutional review boards (MERG Pediatric Cohort, CAAE 52803316.8.0000.5192; MERG Pregnant Women Cohort, CAAE 53240816.4.0000.5190; MERG Case-Controlled Study 51849215.9.0000.5190). Participants were evaluated between February 2017 and August 2019 at two tertiary care hospitals (the Hospital Universitário Oswaldo Cruz and at the Rehabilitation Center of the Fundação Altino Ventura) in Recife, Pernambuco, Brazil.

From a total of 608 children followed in the MERG Pediatric Cohort, SWYC screening information was unavailable in 80 participants who either could not be assessed by SWYC or had inconsistencies in completing the form. Of the remaining 528 children screened with SWYC, a further 254 normocephalic children were not included as their mothers had no laboratory testing for ZIKV by PCR. Therefore, the analysis was conducted on the 274 children who completed neurodevelopmental screening between February 2017 and August 2019 and whose mothers ZIKV status during pregnancy was molecularly confirmed. (Figure 1)
Children were allocated into 4 groups based on their head circumference (HC) measured near the time of the SWYC assessment. For groups 3 and 4, additional data on maternal ZIKV infection status collected during pregnancy and at the time of birth were used to further classify children’s prenatal ZIKV exposure status.

The children in Groups 1 and 2 were born during the peak of the microcephaly epidemic and referred for further neuro-pediatric evaluation as part of their tertiary health care for CZS. Group 1 consisted of 114 children with severe microcephaly and other clinical and/or radiological abnormalities consistent with CZS. Severe microcephaly was defined as a HC corresponding to $\geq 3$ standard deviations (SD) below the mean for sex and age (corrected for prematurity), according to Intergrowth charts. Group 2 included 20 children with moderate microcephaly and other clinical or radiological abnormalities consistent with CZS. Moderate microcephaly was defined as a HC between 2 and $<3$ SD below the mean.

Group 3 included 94 children without microcephaly born to mothers who tested positive for ZIKV during pregnancy by one-step reverse-transcription polymerase chain reaction (RT-PCR) using primers and probes previously described by Lanciotti and et al. Detailed descriptions of the laboratory testing during pregnancy have been previously published. Group 4 included 46 neurotypical children with no microcephaly nor any other brain abnormalities detectable by brain ultrasound at birth who were born to mothers with no laboratory evidence of ZIKV infection during pregnancy. The children in Group 4 were recruited at birth as the control group of a case-control study of microcephaly, whose detailed descriptions of ZIKV testing and clinical evaluations have been previously published.

**Screening Assessment and Clinical Evaluations**

Trained health professionals evaluated children using the SWYC, a standardized instrument for surveillance of children’s neurodevelopment and behavior, translated into Brazilian Portuguese and validated for use in Brazil. The SWYC includes: a) milestones for screening cognitive, language, and motor development in children under 60 months of age, b) the Baby Pediatric Symptom Checklist (BPSC) for social/emotional screening of children under 18 months of age and c) the Preschool Pediatric Symptom Checklist (PPSC) for social/emotional screening of children aged 18 to 60 months. If a child was administered SWYC more than once during follow-up, we analyzed only the results from the oldest age at screening. Similarly, in the case of repeated neurological evaluations, we used the data from the evaluation that was closest to the SWYC assessment.

For the milestones, each form is age-specific (with corrected age for children born prematurely) and includes 10 items. Children were considered at “risk of developmental delay” when their total score for the milestones, as calculated on the scoring sheet, fell below the cut-off points for the respective age groups. As a sensitivity test, results were calculated using the standardized scoring thresholds for both standardized to the original test (USA) and the Brazilian version. For the behavioral evaluations, children were considered to have “suspected behavior abnormalities” if they scored $\geq 3$ on any domains of the
BPSC (i.e., irritability, inflexibility, and difficulty with routines) or ≥9 for the PPSC. Parents were informed of the screening outcomes and received guidance on appropriate home stimulation.

Following initial observations that many of the children with microcephaly did not perform any skills predicted by their chronological age, we adapted the SWYC milestone form to explore the extent of the delays. Specifically, we listed the SWYC milestones from 1 to 36 months of age in increasing order of complexity and evaluated children’s abilities to perform the ranked skills. Assessment using the adapted form was brought to an end after six answers of “not yet.”

Neurologic assessments were performed by qualified pediatric neurologists. Neurological impairment was identified as the occurrence of abnormalities in consciousness, irritability, tone, muscular trophism, muscular strength, and/or proprioceptive reflexes. Radiological abnormalities were detected by brain ultrasound, computed tomography (CT), and/or magnetic resonance imaging (MRI) and included the presence of calcifications, ventriculomegaly, cortical or cerebellar atrophy, and/or neuronal migration disorder.

Data Management and Statistical Analysis

We used the chi-squared test to compare the distribution of participants’ characteristics (sex, gestational age, birthweight, small-for-gestational age, neurologic abnormalities identified by clinical evaluation, and neuroimaging abnormalities) across Groups 1 to 4. We reported the overall statistical significance (p-value), but also calculated the residuals to identify the cells making the greatest contribution to significant results. We calculated the Chi-squared test for trend to compare the proportions of children “at risk” for neurodevelopmental delays and behavioral symptoms across the four groups.

To evaluate skill performance by age in children with severe microcephaly (Group 1), we applied the adapted milestone form in two age subsets: children evaluated at 24 months of age and younger versus children older than 24 months. The non-parametric Mann-Whitney U test was used to compare differences in the performance of development milestones, measured as ordinal scores on the SWYC adapted form. This test takes into account the mean rank of the scores estimates and the corresponding statistical significance.

All findings were considered significant at α=.05; testing was 2-tailed. Data were double entered into the secure digital platform. Analyses were performed using Stata, version 15 statistical software (StataCorp LP) and R software.

Bias and Sample Size

Efforts were undertaken to mitigate the potential for bias in this study. First, the field workers were trained to uniformly apply the SWYC. For normocephalic children, the staff were blinded to information about mothers’ gestational and ZIKV testing history; however, for children with microcephaly (Groups 1 and 2), a blinded assessment was not possible. Second, this study utilizes a validated translation of SWYC to minimize risks of cross-cultural biases and to achieve reliable and comparable measures of the
developmental and behavioral domains. Nevertheless, some potential bias remains. As cases of microcephaly were identified during the emerging epidemic of microcephaly, there is potential for selection bias as it was not possible to assure that microcephaly was representative of all cases born during the Zika outbreak. Specifically, the moderate cases of microcephaly that are less readily clinically detectable could be underrepresented in this cohort. In addition, a survival bias related to CZS severity cannot be excluded as the neurodevelopment assessments were performed at different ages across the clinical groups.

A total of 274 infants underwent neurodevelopmental and behavioral assessment. As this is the first study to use SWYC assessment, there are no previous data to inform the sample size calculations. Therefore, we enrolled all children who fulfilled the inclusion criteria.

**Results**

The study sample comprised 274 children evaluated using SWYC at a mean age of 31.4 months. The HC of the children with microcephaly (Groups 1 and 2) ranged from -2.03 to -10 SD below the mean for age and sex.

Table 1 shows that severe microcephaly cases (Group 1) had a statistically significant higher frequency of abnormal neurological findings (112 of 114 [98.2%]). Similarly, neuroradiological alterations (103 of 106 [97%]) were elevate in Group 1 as compared to moderate microcephaly cases (Group 2) (6 of 16 [37.5%]) and normocephalic children born to ZIKV-positive mothers (Group 3) (5 of 43 [11.6%]). Other clinical findings in Group 1 included: pyramidal syndrome (i.e., defined by the presence of hypertonia, clonus, hyperreflexia and increased archaic reflexes) (89.2%), inadequate responses to visual stimuli (57%), and inadequate responses to auditory stimuli (14%). The frequency of seizures differed across the ZIKV-exposed groups and were reported 67.9% [72 of 106] of the children in Group 1, 17.6% [3 of 17] in Group 2, and 2.2% [2 of 90] in Group 3 (data not shown; the number of children in the groups varied due to missing information on seizures).

For Group 1, SWYC screening indicated risks of development delay in 99% of the children using the Brazilian threshold (100% using the American threshold). Strikingly, none of the children in Group 1 were able to perform skills expected for their chronological age. For Group 2, SWYC screening indicated risks of developmental delay in 65% of the children using the Brazilian threshold (70% using the American threshold). Of note, Group 3 and Group 4 (neurotypical controls) had similar frequencies of children at risk of development delay. Overall, there was a clear gradient of risk (chi-squared test for trend, df=3, p<0.01), with a greater proportion of children at risk of developmental delay in the microcephalic groups compared to the normocephalic groups. Results of the behavior evaluations with the BPSC and PPSC are presented together (i.e., independently of age group) due to the small number of children evaluated under 18 months of age. Based on the BPSC and PPSC, there were no significant differences between the four groups in the frequency of children with “at risk” behavior (Table 2).
Among the Group 1 children assessed for developmental milestones using the adapted SWYC milestone form, 73% (82 of 111) were evaluated after two years of age. Almost all children scored lower than expected, independent of the age of assessment. There were no statistically significant difference in scores by age of assessment except for the milestone of “Laughs” (Table 3).

Discussion

Prior studies have highlighted both the urgent need for further investigation of neurodevelopmental outcomes in children with prenatal ZIKV exposure and the challenge of identifying instruments appropriate for evaluating children across the spectrum of CZS, especially those with severe microcephaly.\textsuperscript{17-19}

In this investigation, we applied the SWYC screening test to assess the neurodevelopment and behavior of prenatally ZIKV-exposed children with and without microcephaly who were born during the ZIKV microcephaly epidemic (2015-2017) in Pernambuco in the Northeast of Brazil.\textsuperscript{20}

According to the SWYC screening, virtually all participants with severe microcephaly (Group 1) and approximately two-thirds of participants with moderate microcephaly (Group 2) were considered “at risk of development delay.” In comparison, 21.7% of ZIKV-exposed normocephalic children (Group 3) and 13.8% of control group children (Group 4) were identified by SWYC assessment as being “at risk.”

The high frequency of “risk of development delay” observed in children with microcephaly is likely attributable to the severity of the cerebral damage. Cerebral malformations generally indicate a poor prognosis in terms of neurodevelopmental function.\textsuperscript{21} In a child with microcephaly caused by etiologies other than ZIKV, the risk of intellectual disability has been estimated to be 10.5% for HC between -2 and -3 SD, 51.2% for HC between -3 and -4 SD, and 100% for HC below -4SD.\textsuperscript{22}

In this cohort of children with ZIKV-related microcephaly, the majority of cases in Group 1 had marked chronic encephalopathy and extensive intraparenchymal cortical calcifications, among other neuroimaging abnormalities. Furthermore, the frequency of central nervous system malformations, pyramidal syndrome, epilepsy, inadequate response to visual and auditory stimuli were higher in Group 1 than in the other groups evaluated. These neuroimaging and clinical findings are predictors of severe neuropsychomotor impairment and are among the phenotypic characteristics of CZS.\textsuperscript{1, 2, 23-25}

Consistent with our findings, a 2019 Brazilian investigation assessing children with cerebral palsy and probable CZS, of whom 97.5% had microcephaly, using the Bayley Scale of Infant and Toddler Development III (Bayley-III) reported scores below 70 (i.e., suggesting severe developmental delays) for almost all participants across all three scales: cognitive, 95.1%; language, 97.6%; motor, 97.6%.\textsuperscript{23}

A case series study assessing 24 children with ZIKV-related microcephaly in Northeast Brazil using the Denver Developmental Screening Test II also found a high degree of impairment for neuropsychomotor development. The study reported that children with a mean age of 19.9 months scored, on average,
development milestones equivalent of ages 2.1 to 3.4 months, across the domains of language, motor, and personal/social skills.\textsuperscript{26}

Microcephaly, of any severity, is considered a useful indicator for developmental delays. However, in our study we compared neurodevelopment in children with severe and moderate microcephaly and observed, that among children with moderate microcephaly, 35% "Appear to Meet Age Expectations" using the SWYC assessment. Therefore, this strategy of classifying the microcephaly into moderate or severe allowed us to observe that the predictive value of SWYC varies according to the severity of microcephaly.

Although the frequency of cases with moderate microcephaly in our sample “at risk of developmental delay” was lower than the frequency in cases with severe microcephaly, the percentage of “at risk” children was higher than that found in the normocephalic groups, which included the ZIKV-exposed and control children. Indeed, both normocephalic groups had similar frequencies of “at risk” children to each other and to the percentage of “at risk” children expected to be found with screening tests in the general population.\textsuperscript{27}

Prior to this study, few studies with comparable methods have investigated the development of children without microcephaly who were exposed to ZIKV prenatally. In a cohort in the Southeast Brazil that was assessed using Bayley-III, 28% of ZIKV-exposed children presented with at least one below average score (i.e., scores <85-70) for cognitive, language, and motor function.\textsuperscript{28} In using a screening test instead of a more comprehensive developmental assessment, such as Bayley-III, we would expect an even higher percentage of children identified as being at risk of developmental delay; however, our results from the SWYC screening suggest a lower frequency of children at risk of developmental delay in this cohort than compared to the Rio de Janeiro sample. Nevertheless, we note that a normal SWYC test cannot exclude subsequent later-onset neurodevelopment repercussions. Therefore, we recommend that children with prenatal ZIKV exposure should undergo a longitudinal evaluation, using additional and more accurate and comprehensive tests, such as the Bayley-III.\textsuperscript{29}

The adapted SWYC form made it possible to observe that children with severe microcephaly were severely limited in their ability to achieve developmental milestones that were appropriate for their chronological age. Even though over 74\% of the children assessed were >24 months of age, over 80\% were unable to perform tasks corresponding to the expected skill acquisition for 5-8 month of age, such as item 14 of the adapted form ("passes a toy from one hand to another"). When children were divided into two groups (14-24 months vs. 25-32 months), we observed no differences in the achievement of developmental milestones, with the exception of the item indicating “Laughs”. When comparing the performance of the older and younger groups, these findings suggest a significant limitation in the ability of the children with severe microcephaly to achieve new milestones as they get older, which may be explained by the severity of their neurological impairment.

The adapted form not only enabled the detection of this delay, but also allowed us to describe of the deficit profile, which has the potential to inform earlier and more targeted multidisciplinary intervention to
address the identified needs. Although the SWYC tool was not specifically designed for this purpose, this study demonstrates an additional application of the SWYC, which addresses the current lack of specific instruments for evaluating development in children with severe neurological impairment. Further follow-up studies and repeated measures will be valuable for confirming the observation that children with severe ZIKV-related microcephaly achieve developmental milestones corresponding to lower chronological ages than their own.

Using the SWYC checklists, the risk for behavioral and emotional symptoms was observed to be similar among the groups. It is plausible that the SWYC questions related to behavior and emotional symptoms may have generated inconsistent responses for children with severe microcephaly, due to the children’s serious motor and intellectual limitations. Questions such as "Is your child interested in playing with other children", "Does your child break things on purpose", or "Is your child fidgety or unable to sit still" are likely out of context for most children with severe microcephaly. Therefore, we suggest that these results do not mean that Group 1 has lower risk for behavioral problems, but rather that children with severe microcephaly do not possess the cognitive, emotional and motor skills required to demonstrate the “at risk” behaviors. Therefore, this result should be interpreted with caution.

Initial descriptions of CZS mentioned irritability as a frequent clinical finding. Although this characteristic was often reported and observed in newborns and young infants with a phenotype typical for CZS, irritability became less evident as the children grew (personal observation of the authors). In this investigation, irritability was not a predominant complaint in Group 1, which may be related to the fact that most of these children were assessed after the second year of life, at which time irritability may have been less likely to be presented. It is not possible to determine whether the condition was resolved or if this behavior was modified by the frequent use of anticonvulsants in this population.

Also unexpectedly, children without microcephaly (Groups 3 and 4) demonstrated high frequencies of risk signs of behavioral and emotional symptoms (42.5% and 63.7%, respectively). Studies worldwide have shown a progressive increase of in the prevalence of behavioral abnormalities in childhood. In our study, it is possible that the low level of parents’ educational attainment may have limited the infants’ development of social and emotional skills, thereby generating a high frequency of “at risk” behavior.

Conclusions

Children with prenatal exposure to ZIKV may manifest different levels of neurodevelopmental impairment, and even amongst children with microcephaly, there is a variability in functional performance. Our results suggest that children with severe microcephaly do not seem to acquire new skills beyond a certain stage of development. To confirm these findings, we suggest that children with severe microcephaly should be evaluated with repeated measurements from the adapted form. In addition, our results provide evidence that children prenatally exposed to ZIKV without microcephaly are similarly likely to be at risk of development delay as unexposed children in the control group. For children with prenatal exposure to ZIKV at risk of developmental delay, we suggest repeated neurodevelopmental
assessments using more accurate and comprehensive instruments, such as the Bayley-III. SWYC may be adopted as a screening tool, thereby enabling at-risk children to be referred for further detailed assessment and multidisciplinary care.

**Abbreviations**

BPSC: Baby Pediatric Symptom Checklist  
CT: Computed Tomography  
CZS: Congenital Zika Syndrome  
HC: Head Circumference  
MERG: Microcephaly Epidemic Research Group  
MRI: Magnetic Resonance Imaging  
PCR: Polymerase Chain Reaction  
PPSC: Preschool Pediatric Symptom Checklist  
RT-PCR: reverse-transcription polymerase chain reaction  
SD: Standard Deviations  
USG: Ultrasonography  
SWYC: Survey of Wellbeing of Young Children  
ZIKV: Zika Virus

**Declarations**

**Ethics approval and consent to participate:** Protocols were approved by the institutional review board at Fiocruz-PE and Universidade de Pernambuco (UPE) and was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation guideline for Good Clinical Practice, and the codes and regulations of Brazil regarding research on human subjects. Written informed consent was obtained from parents or guardians. The study protocols are available at the MERG website.

**Consent for publication:** Does not apply.

**Availability of data and materials:** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
Competing interests: The authors declare that they have no competing interests.

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Authors’ contributions: PFSS, SHE, MDCGC, RAAX, DBMF conceptualized and designed the study, designed the data collection instrument, coordinated and supervised data collection. PFSS, SHE, FCLSPG, MCL, WVS and URM performed the statistical analysis and interpretation of data. PFSS and SHE drafted the initial manuscript. DBMF, RAAX, MCL, CMTM, TVBA, EBB and LCR critically reviewed and revised the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Birth characteristics, neurological and radiological characteristics of groups of infants with severe and moderate Zika related microcephaly, as well as those exposed and normal controls.

| Biological and radiological characteristics | Study Groups | P Value |
|---------------------------------------------|--------------|---------|
|                                             | Group1 | Group2 | Group3 | Group4 |     |
|                                             | Severe Microcephaly (n = 114) | Moderate Microcephaly (n = 20) | ZIKV maternal infection (n = 94) | Controls (n = 46) |     |
| Sex, No. (%)                               | Female | 64 (56.1) | 12 (60.0) | 47 (50.0) | 25 (54.3) | .786 |
|                                             | Male   | 50 (43.9) | 8 (40.0)  | 47 (50.0) | 21 (45.7) |     |
| Prematurity, Weeks of Gestational Age, No. (%) | <37   | 16 (14.2) | 4 (25.0)  | 6 (7.9)   | 7 (15.2)  | .249 |
|                                             | ≥37    | 97 (85.8) | 12 (75.0) | 70 (92.1) | 39 (84.8) |     |
| Birthweight, g, No. (%)                     | <2500  | 28 (25.5) | 10 (58.8)c | 5 (7.1)   | 2 (4.3)   | <.001 |
|                                             |       | 47 (42.7) | 3 (17.6)  | 14 (20.0) | 12 (26.1) |     |
|                                             | ≥3000  | 35 (31.8) | 4 (23.5)  | 51 (72.9) | 32 (69.6) |     |
|                                             | Missing | 4 | 3 | 24 | 0 |     |
| Small-for-gestational age, No. (%)          | Yes | 29 (26.4) | 8 (47.1)c | 5 (7.1)   | 2 (4.3)   | <.001 |
|                                             | No    | 81 (73.6) | 9 (52.9)  | 65 (92.9) | 44 (95.7) |     |
|                                             | Missing | 4 | 3 | 24 | 0 |     |
| Clinical neurological abnormalities, No. (%) | Yes | 112 (98.2)c | 12 (60.0) | 10 (11.1) | 3 (7.5)   | <.001 |
|                                             | No    | 2 (1.8)   | 8 (40.0)  | 80 (88.9) | 37 (92.5) |     |
|                                             | Missing | 0 | 0 | 4 | 6 |     |
| Neuroimaging abnormalities, No. (%)         | Yes | 103 (97.2)c | 6 (37.5)  | 5 (11.6)  | 0 (0)     | <.001 |
|                                             | No    | 3 (2.8)   | 10 (62.5) | 38 (88.4)d | 46 (100) |     |
|                                             | Missing | 8 | 4 | 51 | 0 |     |
The number of children varied due to missing values

a Normocephalic children born to mothers ZIKV PCR+

b Normocephalic children, considered neurotypical controls.

c Statistically significant higher observed frequency than expected

d For Group 3 brain ultrasound was performed within the first 6 months of life. For children who had the first evaluation over 6 months, neuroimaging was performed only according to clinical indication.

Table 2. SWYC neurodevelopmental and behavioral outcomes in children with severe and moderate Zika related microcephaly, children exposed without microcephaly and normal controls.

| SWYC components | Study Group | P Value |
|----------------|-------------|---------|
|                | Severe Microcephaly (n = 114) | Moderate microcephaly (n = 20) | ZIKV maternal infection (n = 94) | Controls (n = 46) |
| Development milestone (Brazil), No. (%) | | | | |
| “At risk”/Needs review | 113 (99.1)c | 13 (65.0) c | 13 (13.8) | 10 (21.7) | < .001 |
| “Appears to Meet Age Expectations” | 1 (0.9) | 7 (35.0) | 81 (86.2) | 36 (78.3) | .001 |
| Development milestone (USA), No. (%) | | | | |
| “At risk”/ Needs review | 114 (100.0) c | 14 (70.0) c | 19 (20.2) | 12 (26.1) | < |
| “Appears to Meet Age Expectations” | 0 (0.0) | 6 (30.0) | 75 (78.8) | 34 (73.9) | .001 |
| Baby and Preschool Pediatric Symptoms Checklist, No. (%) | | | | |
| “At risk”/Needs further evaluation or investigation | 55 (51.0) | 13 (65.0) | 40 (42.5) | 28 (63.7) | .70 |
| Adequate | 53 (49.0) | 7 (35.0) | 54 (57.5) | 16 (36.3) |
| Missing | 6 | 0 | 0 | 2 |

a Normocephalic children born to mothers ZIKV PCR+

b Normocephalic children, considered neurotypical controls.

c Statistically significant higher observed frequency than expected

Table 3. SWYC Adapted Form comparing children with Severe Zika related Microcephaly divided by age groups
| DEVELOPMENT MILESTONES                                      | ≤ 24 MONTHS (n=29) | > 24 MONTHS (n=82) | P Valuea |
|-------------------------------------------------------------|---------------------|--------------------|----------|
| Makes sounds that let you know he or she is happy or upset | Not yet 0 6 23 53.5 | Not yet 5 6 71 56.9 | .44      |
| Seems happy to see you                                     | Some what 1 2 26 54.8 | Some what 2 4 76 56.4 | .61      |
| Follows a moving toy with his or her eyes                   | Very much 5 10 14 50.3 | Very much 7 26 49 58.0 | .21      |
| Turns head to find the person who is talking                | Mean Rank 53.5       | Mean Rank 76.1      |          |
| Holds head steady when being pulled up to a sitting position|                     |                    |          |
| Brings hands together                                       |                     |                    |          |
| Laughs                                                      |                     |                    |          |
| Keeps head steady when held in a sitting position           |                     |                    |          |
| Makes sounds like "ga," "ma," or "ba"                      |                     |                    |          |
| Looks when you call his or her name                         |                     |                    |          |
| Rolls over                                                  |                     |                    |          |
| Passes a toy from one hand to the other                     |                     |                    |          |
| Looks for you or another caregiver when upset               |                     |                    |          |
| Holds two objects and bangs them together                   |                     |                    |          |
| Holds up arms to be picked up                                |                     |                    |          |
| Gets into a sitting position by him or herself              |                     |                    |          |
| Picks up food and eats it                                   |                     |                    |          |
| Pulls up to standing                                        |                     |                    |          |
| Plays games like "peekaboo" or "patacake"                  |                     |                    |          |
| Calls you "mama" or "dada" or similar name                 |                     |                    |          |
| Looks around when you say things like "Where's your bottle?" or "Where's your blanket?" | | | |
| Copies sounds that you make                                 |                     |                    |          |
| Walks across a room without help                            |                     |                    |          |
| Follows directions like “Come here” or “give me the ball”  |                     |                    |          |
| Runs                                                        |                     |                    |          |
| Walks upstairs with help                                    |                     |                    |          |
| Kicks a ball                                                |                     |                    |          |
| Names at least 5 familiar objects like ball or milk         |                     |                    |          |
| Names at least 5 body parts like nose, hand, or tummy      |                     |                    |          |
| Climbs up a ladder at a playground                          |                     |                    |          |
| Uses words like “me” or “mine”                              |                     |                    |          |
| Jumps off the ground with two feet                          |                     |                    |          |
| Puts 2 or more words together like “more water” or “go outside” | | |          |
| Uses words to ask for help                                  |                     |                    |          |

Notes:
- P Valuea: Statistical significance of the difference between the two groups.
Figures

Figure 1

Flow diagram of children enrollment