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Infant neurodevelopment and behavior in Guadeloupe after lead exposure and Zika maternal infection during pregnancy

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Authors' contributions

Study conception: GM, BH, LMu, PQ, CC, SC

Data collection, quality control: MK, LMi, BT

Chemical analysis: BLB

Data analysis: MK, CC, SC

Writing – original draft: MK, GM, CC, SC

Writing – review editing: MK, GM, LMi, BLB, BH, BT, LMu, PQ, CC, SC

All authors have read and approved the final manuscript.

Abstract:

Background

Prenatal lead exposure is known to have neurotoxic effects on the developing fetus, while some viral infections may have a tropism for the central nervous system. Our objective was to study whether the effects of prenatal lead exposure on infant development and behaviors at 18 months of age are modified by the occurrence of a maternal infection to Zika virus (ZIKV) during pregnancy.

Methods

During the ZIKV epidemic in Guadeloupe in 2016 a cohort of pregnant women was set up. Blood samples (pregnancy, childbirth and cord) (n=297) enabled us to measure blood lead levels aimed to determine prenatal lead exposure and the likelihood of maternal infection during pregnancy (ZIKV status + vs -). The 18 months “Ages and Stages Questionnaire” (ASQ) was used to generate scores for global development, fine and gross motor skills, communication, problem solving, and personal-social skills. The questions from a longitudinal cohort study conducted in Canada (Québec) were used to generate hyperactivity, opposition, inattention and physical aggression scores. Associations were tested by multivariate linear regressions.

Results

Prenatal lead exposure was associated with delays in neurodevelopment at 18 months, reflected by lower scores in ASQ totals, and in the fine motor and problem-solving domains. Some of these associations appeared to be sex-specific, observed almost exclusively in boys (ASQ total, fine motor and personal-social scores). Prenatal lead exposure was not associated with behavioral scores. ZIKV infection during pregnancy was associated with a lower fine motor ASQ score, and higher scores for hyperactivity, opposition and physical aggression. Significant interaction between prenatal lead exposure and ZIKV status was observed with a lower personal-social score in ZIKV (-) only, and for hyperactivity and inattention scores, though some of these interactions (ASQ personal-social score, inattention score) were no longer significant when children with microcephaly were excluded from the analyses.

Discussion/Conclusion

Our study confirms previous findings of associations between prenatal exposure to lead at low levels and adverse neurodevelopmental outcomes during infancy and the particular vulnerability of boys. It suggests associations between ZIKV infection during pregnancy and adverse effects on a number of neurodevelopmental functions (fine motor function) and behaviors (opposition, hyperactivity), that need to be confirmed at later age. There is no strong evidence of interaction between ZIKV infection and lead exposure but both prenatal risk factors may affect fine motor function.

Keywords : Prenatal exposure, lead, Zika, infancy, development, behavior, biomarkers

Introduction

The neurotoxic potential of early life exposure to lead is well established and empirical evidence suggests that there is no safe level for exposure occurring during infancy and childhood. Exposure to lead during these developmental periods has consistently been associated with subtle deficits in important child outcomes such as cognitive ability, including intellectual quotient (IQ), attention, memory and problem solving, as well as visuo-spatial skills, motor abilities and regulation (Crump et al. 2013; Gagnon-Chauvin et al. 2020; Lanphear et al. 2005) at blood levels below 7.5 and even 5 $\mu\text{g}/\text{dL}$, with a linear exposure-response curve (McFarland et al. 2022), steeper at low levels than at higher levels. Recently, one systematic review and one meta-analysis both concluded that there are significant associations between low levels of lead exposure during childhood and Attention Deficit/Hyperactivity Disorder (ADHD) symptoms and diagnosis (Donzelli et al. 2019; He et al. 2019).

The developmental effects of prenatal lead exposure have been studied less, mostly because this would require a longitudinal research design including maternal enrollment during pregnancy or at delivery, for characterization of prenatal exposure. Most studies have reported lower IQ among children exposed prenatally to very low levels of lead (below 5 $\mu\text{g}/\text{dL}$) (Desrochers-Couture et al. 2018; Fruh et al. 2019; Jedrychowski et al. 2009; Polanska et al. 2018; Tatsuta et al. 2020), with the exception of the UK birth cohort (Taylor et al. 2017). Several of these studies observed these associations only in boys (Desrochers-Couture et al. 2018; Polanska et al. 2018; Tatsuta et al. 2020). Alterations in behavior (such as attention deficits or hyperactivity) have also been reported in association with prenatal exposure to lead at low levels (Neugebauer et al. 2015; Sioen et al. 2013).

Recently, the need to consider multiple exposures occurring at various periods in life (especially prenatally) has been recognized as a more comprehensive risk assessment. A large number of studies of children's neurobehavior have been published recently, evaluating the role played by mixtures of neurotoxic agents or potential modifiers (Bauer et al. 2020; Vuong et al. 2020). Exposure to mixtures of metals including lead in particular has been studied using novel statistical methodology (Lazarevic et al. 2019), with the objective of estimating the overall effect of the mixture and identifying the agents in the mixture responsible for the health effects as well as the potential joint effects of some of these agents (Freire et al. 2018; Guo et al. 2020; Valeri et al. 2017). In these studies, lead is usually identified as an active component of the mixture, sometimes in interaction (such as with arsenic – (Freire et al. 2018)). Other modifiers of lead's toxic action have been suggested in addition to sex (previously mentioned). For example, maternal stress during pregnancy was reported as an effect modifier of prenatal lead neurotoxicity (Tamayo et al. 2017) on cognitive development during infancy, as was prenatal tobacco's interaction that was reported as an effect modifier with childhood lead exposure in association with ADHD (Froehlich et al. 2009).

To our knowledge, no joint effect of lead exposure and viral exposure has ever been reported in humans. Zika virus (ZIKV) is a mosquito-borne flavivirus. ZIKV can cross the placenta, and is the only arbovirus linked to congenital malformations. An estimate of 4 to 9% of women with a prenatal ZIKV infection result in suspected congenital ZIKV syndrome (CZS) including brain and ocular malformations, microcephaly, congenital deafness, limb contractures, and arthrogryposis (Adams et al. 2016; Krauer et al. 2017; Melo et al. 2016; Oliveira Melo et

al. 2016; Pomar et al. 2019; Rasmussen et al. 2016; Valentine et al. 2016; van der Linden et al. 2016). The study of the health consequences of co-exposure to lead and biological agents can be justified (especially when these agents share modes of action) in order to identify whether synergetic processes exist. In the fetus, the principal targets of ZIKV are neural progenitor cells (NPC) and glial cells in the developing brain. The consequences are cell death and reduced NPC proliferation, which results in impaired brain development (Wen et al. 2017). In parallel Huang and Schneider, suggest that even low levels of lead can differentially affect the proliferation, migration and differentiation of embryonic neural stem cells originating from different brain regions (Huang and Schneider 2004). Other action mechanisms include alterations in neurotransmissions (especially serotonin and catecholaminergic neurotransmission in the cerebral cortex, hippocampus, and cerebellum), and disrupting mechanisms of the endocrine hypothalamic–pituitary–gonadal (HPG), hypothalamic–pituitary–thyroidal (HPT), and hypothalamic–pituitary–adrenal (HPA) axes (Gagnon-Chauvin et al. 2020).

A ZIKV epidemic started in Guadeloupe (French West Indies) in April 2016; the epidemic peak was reached during the first week of June. In total, it lasted 22 weeks, with more than 30,500 clinically suggestive cases (SpF 2017) (nearly 10% of the population) including 639 cases biologically confirmed in pregnant women for whom, in January 2017, eight microcephaly and five other fetal brain malformations had been detected on ultrasound (SpF 2017). The present study was set up during the ZIKV epidemic to study whether prenatal ZIKV and lead, and their co-exposure, are related to non-optimal neurodevelopment and behaviors during infancy.

Materials and Methods

Population and data collection

The ZIKA-DFA-FE/BB studies were carried out in the French Territory of America (which includes the islands of Guadeloupe, Martinique, Saint-Martin, and Saint-Barthelemy as well as the territory of French Guiana in South America) during the 2016 ZIKV epidemic, they sought to address the natural history of ZIKV infection during pregnancy (Hoen et al. 2018). Cohorts of pregnant women and their infants were monitored from birth to the age of 24 months, and this monitoring included the collection of clinical and paraclinical information (including ultrasounds and biological results) within the framework of usual care practices, as well as the constitution of a biological collection for determination of ZIKV infection during pregnancy. These studies received ethical approval from the *Comité de Protection des Personnes Sud-Ouest et Outre-Mer III*. All participants provided written informed consent. Both cohorts were implemented by the CIC 1424 Inserm.

The present study is ancillary to the ZIKA-DFA-FE cohort study. Eligible women were those who delivered live newborns at the maternity ward of the University hospital of Guadeloupe after 14 October 2016, starting on the day of this complementary study. A total of 740 women (of 837) were enrolled in the present study, and for most a maternal face-to-face interview was conducted at the maternity unit after delivery (n=684), or when they left the hospital via a phone interview (n=25) or a home visit (n=31). These interviews were conducted by research staff trained in the collection of socioeconomic and demographic characteristics. Blood samples were collected within the framework of the ZIKA-DFA-FE study. Data on child development and behavior, maternal lifestyle and socioeconomic characteristics were obtained from the 421 families participating in the 18-month follow-up via an interview conducted by phone (n=300), or in person at the hospital (n=119) or at home (n=2).

435 women had blood samples available for metals assessment, and of these, 303 women were followed up after 18 months. A posteriori exclusion criteria included maternal conditions during pregnancy and those pregnancy characteristics very likely to impact infant development – such as a history of human immunodeficiency virus infection (n=2) or preterm delivery (very and extremely premature babies) (n=4). Our study is based on 297 mother-child dyads at the 18-month follow-up (**Figure 1**).

Assessment of prenatal exposure to lead and other metals

A maternal blood sample (10 ml of peripheral venous blood in EDTA tubes) was collected at delivery. An aliquot of whole blood was taken, then the rest was centrifuged to collect the supernatant (plasma). Whole blood and plasma were temporarily stored at -30°C by the Centre of Biological Resources at the University Hospital of Guadeloupe. These blood samples were sent to the LERES laboratory in Rennes in UN3373 packaging, under frozen temperature control (-80°C).

Eight elements (Pb, Mn, Cu, Zn, Cd, As, Se and Hg) were measured in 435 whole blood samples. For each sample, a 0.5 ml volume of 67% acid was added to 0.5 ml of blood prior to a mineralization step at 83°C lasting 4 hours, using a graphite block (Hotblock Pro, model SC-189, Environmental Express). The mineralized samples were diluted 1:20 with a solution of HCl (2% in ultrapure water), and analyzed by a plasma torch coupled to a tandem mass spectrometer (ICP-MSMS 8800, Agilent Technologies). For the determination of selenium and arsenic, interferences were managed using a tandem mass spectrometer and the oxygen mode. On-line addition of internal standards (Sc, Ge, ⁷⁷Se, Rh, Re and Ir) was performed to correct for matrix effects and verify the absence of drift (signal variation less than 25%). To resolve interference effects, Se quantification was performed by isotopic dilution with ⁷⁷Se.

For each analytical sequence, consumable blanks and reference blood tests were systematically analyzed (lyophilized blood: Utak level 1; Utak level 2). The results of the blood samples were validated if the concentrations of the reference blood at each level were within the limits of the control cards. The measurement uncertainties for each element in the control samples were all less than 20%. The limits of quantification (LOQ) for Pb, Mn, Cu, Zn, Cd, As, Se and Hg were 2, 5, 200, 200, 0.25, 1, 20 and 0.4 µg/L respectively. The laboratory participated in the interlaboratory tests organized by the INSPQ (Institut National de Santé Publique du Québec) on the determination of metals in blood (obtaining a Z score < 3), which enabled the method to be accredited by the French accreditation committee (COFRAC 2019). All metals compounds were detected with percentages of above 70%. An imputation method was applied for missing data (i.e. <LOD) using a maximum likelihood method (Jin et al. 2011). Then the lead concentration was log-transformed to the base of ten, to satisfy normality and have interpretable estimates.

Definition of the ZIKV infection during pregnancy

Since ZIKV infection can be asymptomatic (or when it results in clinical signs these are non-specific) the definition of a ZIKV+ case required biological confirmation. For molecular diagnostics, the “window” during which it is possible to reveal the ZIKV genome by Polymerase Chain Reaction in the blood or in the urine is narrow: between 7 and 10 days from the date of the start of clinical signs (HAS 2016). Therefore, all women included in the ZIKA-DFA-FE cohort benefited from an anti-ZIKV ELISA serological test (Euroimmun ©, Hully 2016), carried out on a blood sample taken at time of delivery (maternal and cord blood), regardless of their

ZIKV infection status during pregnancy or during delivery (asymptomatic, symptomatic, PCR negative or positive). Confirmed ZIKV+ and ZIKV- cases were defined according to an algorithm used in the ZIKA-DFA-FE cohort, taking into account clinical status, the result of the PCR prescribed if necessary during pregnancy, the ZIKV serology (IgM and / or IgG detection) of maternal or cord blood, the date of initiation of the pregnancy and the ZIKV 2016 epidemic period in Guadeloupe (calendar weeks 17 to 39, that is April 25 to September 25) (Hoen et al. 2018). Where the algorithm was unable to classify women into confirmed ZIKV+ or ZIKV- cases (approximately 40%), we defined “probable ZIKV exposure” according to the date of the beginning of pregnancy, considering the epidemic curve. The epidemic peak having occurred in the 23rd week of 2016, the end of the 27th week (10th of July 2016) was chosen as a cut-off point of exposure to ZIKV during pregnancy. If the date of start of pregnancy preceded the start of the 28th week of 2016, then the woman was classified as a “probable ZIKV-exposed case”. If the date of start of pregnancy came after that week, then she was classified as a “probable ZIKV-unexposed case”. Regarding ZIKV infection status, two variables were then considered: one taking into account confirmed cases only based on the algorithm ZIKA-DFA-FE (in supplemental data; 183 mother-child pairs at the 18-months follow-up); the other combining confirmed and probable cases, which underpins the main results.

Outcomes

Neurodevelopment at 18 months of age was documented using the French version of the Ages and Stages Questionnaire (ASQ); this is a screening test aimed at identifying children at risk of developmental delay (Squires and Bricker 2009). Fine motor, gross motor, personal-social, problem-solving and communication subscales were answered by the mother; each subscale contains six items. Mothers had to answer each item using a 3-point Likert scale ranging from 10: “yes”, 5: “sometimes” to 0: “not yet”. The sum of response items provided total scores for each subscale, and the sum of all five subscale scores provided a total ASQ score. Higher continuous scores are indicators of better development in each domain. Examination of the variable distributions revealed a ceiling effect for the gross motor development subscale, which was therefore dichotomized at the median value of 60. 15 questions from the Quebec Longitudinal Study of Child Development (QLSCD) were used to document the following externalizing behaviors: hyperactivity (6 items), opposition (3 items), inattention (3 items) and physical aggression (3 items) (Orri et al. 2021). These questions were shown to be associated with characteristics known to be risk factors for behavioral impairment, such as prenatal tobacco exposure, family type, maternal age and maternal depression (Ahun et al. 2018; Galera et al. 2011; Huijbregts et al. 2007, 2008). Mothers had to answer each question describing her child’s behavior using a 3-point Likert scale ranging from 1: “never”, 2: “sometimes” or 3: “often”. A higher score denotes more severe behavioral difficulties.

Other data

Birth weight (in g), the date of start of pregnancy and gestational age (weeks of gestation based on the first trimester ultrasound (WG)) were abstracted from medical records. Maternal sociodemographic and lifestyle characteristics were, in most cases, collected via a face-to-face post-delivery interview and included: maternal date of birth, pre-pregnancy height (cm) and weight (kg), parity (primiparous vs. multiparous), place of birth (French West Indies for Martinique / Guadeloupe / Saint-Martin / Saint-Barthelemy; other West Indies for Cuba

/ Dominican Republic; mainland France, rest of Europe and others), family situation (with a partner, alone with family members, alone), education level (≤ 12 years, > 12 years), and smoking status during pregnancy (no, yes). Maternal pre-pregnancy BMI was calculated as weight (kg)/height (m)².

At the 18-month follow-up, the following information was obtained from the maternal interview: breastfeeding duration (number of months); household income (<450€, 451-800€, 801-1500€, 1501-3000€, >3000€); quality of stimulation provided to the child via two indicators, 1) maternal depression symptoms (using the third quartile cutting point (> 12) of the 10-item Center for Epidemiologic Studies Depression Scale (CES-D-10; (Radloff 1977)), and 2) the quality of maternal stimulation provided to the child. This was estimated using five questions: "How often do you sing along with your child?", "How often do you read stories or show pictures in a book to your child?", "Do you play with games with your child at home?", "Do you take your child for a walk at the beach or park?", and "Do you play physical games (ball) with your child?" Mothers had to specify how often they interact with their child in these ways: (3) almost every day, (2) several times a week, or (1) less than once a week or almost never. The sum of responses to the items ranged from 5 to 15, where a higher score suggests greater opportunities for stimulation (Pele et al. 2013).

Statistical analysis

For all scores calculated, where fewer than 50% of items were missing, a correction method was used as follows: $\text{Score} = \sum \text{items of the score} * (\text{total N of items} / \text{N of non-missing items})$. The study population characteristics were described using means, standard deviations and percentages. The determinants of ZIKV infection and blood lead concentrations were studied using Student t, χ^2 and Fisher tests to describe differences between infected/exposed and non-infected/unexposed mothers. Using simple linear and simple logistic models, neurodevelopment and behavior scores obtained at the 18-month follow-up were studied in relation to lead exposure, ZIKV exposure and the interaction of lead exposure and ZIKV exposure. Multivariable linear regression models for continuous outcomes and logistic models for dichotomous outcomes were used.

Covariates were chosen a priori as major risk factors identified in the literature. For infants: sex, birthweight, gestational age and age at 18-month follow-up; for mothers: age at delivery, pre-pregnancy BMI, parity, education level, marital status, tobacco use during pregnancy, breastfeeding duration, mother's stimulation score and depression symptoms. Where the covariates were continuous, a linearity test was used to decide how they should be considered, comparing embedded models using the likelihood ratio test, with the ordinal variable declared as either continuous or categorical. If linearity was not accepted, they were categorized in quartiles. Covariates associated at a p-value of 0.20 or less were included in multiple regression models to assess their confounding influence by removing them one by one, starting with the least significant for the outcomes. For the developmental and behavioral outcomes, sex was forced in the models. Lead concentrations were analyzed both as categorical variables (in tertiles to assess a possible nonlinear association) and as continuous (log to the base of ten). Prenatal exposures to other metals were simultaneously tested ($p < 0.20$) in the regression models, after studying correlation with lead using the Spearman correlation. These were considered possible confounders if the correlation between lead and the compound had a p-value of < 0.01 .

The possible statistical interaction between prenatal ZIKV exposure and lead tertiles was tested assuming an additive or multiplicative scale in linear or logistic regression models. When the interaction term was statistically significant at p -value < 0.20 , the adjusted means (obtained using the “lsmeans” statement from the SAS PROC GLM) of the outcome were presented graphically for each stratum built by ZIKV exposure and lead tertiles. The possible statistical interaction between sex and lead tertiles was tested and was presented in supplementary analysis.

Four series of sensitivity analyses were performed to assess the robustness of the associations observed: (i) The exclusion of children with microcephaly (defined as head circumference less than 2 SD below the mean for sex and gestational age using the INTERGROWTH-21st Z-scores (Villar et al. 2014)) to see if the associations observed were driven by those cases, or not; 18 newborns were classified as microcephalic. (ii) In addition to the complete cases ($n=258$), analyses with imputations to address missing data on demographic and socioeconomic characteristics. The missing values of each variable were less than 9% of the source population (8.5% for maternal depression symptoms; 2.7% for maternal stimulation score; 1.4% for BMI; 0.7% for breastfeeding duration; 0.3% for maternal education level; 0.3% for parity; 0.1% for gestational age at delivery; 0.1% for family situation; and 0% for all other variables of interest). Predicted values of a linear regression with selected covariates ($p < 0.20$) were used to impute the missing data. (iii) The exclusion of infants with those medical conditions most likely to impact infant development (Sickle cell disease ($n=1$), hospitalized infants for convulsion without fever ($n=3$)). (iiii) The exclusion of outliers, identified using DFFITS for linear regression or standardized Pearson chi-square residuals for logistic regression.

Statistical analysis was performed using SAS software (version 9.4). The significance level was set at 0.05.

Results

The sociodemographic and medical characteristics of participating mothers and infants are shown in **Table 1**. The mean age at delivery was 30.5 years of age. Most of the mothers were born in the French West Indies (60.9%), and had completed high school (62.3%). 22% of participating mothers were obese pre-pregnancy, and 9% smoked cigarettes during pregnancy. Lead was detected in 100% of maternal blood samples. The mean concentration level was 13.5 $\mu\text{g/L}$ (median 11 $\mu\text{g/L}$). Tertiles of lead concentration ($\mu\text{g/L}$) were: T1 [2.2 - 9.2], T2 [9.2 - 13.3], T3 [13.3 - 111].

Lead concentrations were mildly correlated with cadmium ($r=0.33$), zinc ($r=0.21$), selenium ($r=0.20$) and manganese ($r=0.21$), and somewhat less related to mercury ($r=0.09$) concentrations. Maternal lead concentrations were positively correlated with age at delivery and pre-pregnancy BMI. Blood lead levels were higher among those women not having completed high school and those born in other West Indies (**Table 1**). Women infected by ZIKV during pregnancy were more likely to be younger at delivery, and to have higher scores for depressive symptoms (**Table 1**). On average, breastfeeding duration was 7½ months, and the mean age of infants at follow-up was 17.4 months (age corrected for gestational age). **Table 2** shows the descriptive statistics of the outcomes under study. Bivariate associations between outcomes of interest and socioeconomic and demographic characteristics are shown in **Supplementary Table 1**.

Table 3 showed that higher tertiles of lead were associated with lower ASQ total scores, fine motor subscale and problem-solving subscale scores. The score decreases were monotonous (ptrend 0.03, 0.02 and

<.0001, respectively) and when using log-transformed lead concentrations, the score decreases were -19.3 [-39.8; 1.4] for ASQ total score, -5.9 [-12.2; 0.5] and -14.6 [-21.7; -7.6] for fine motor and problem-solving scores, respectively. ZIKV exposure was associated with an ASQ fine motor score decrease of -3.5 [-6.3; -0.8], though not with any other ASQ scores. **Table 4** shows a significant interaction between ZIKV exposure and lead exposure for the ASQ personal-social score, while a monotonic decrease in the score with lead exposure was observed only in ZIKV (-) women. The results are presented in **Figure 2** (p -interaction=0.05 with additive scale). No evidence of interaction between ZIKV exposure and lead tertiles was observed for any of the other ASQ scores (**Table 4**). Infant sex and prenatal lead interactions are significant for ASQ total, fine motor and personal-social scores, a monotonic decrease in the scores with lead exposure was observed only in boys (**Supplementary Table 2**).

Table 5 showed no association between maternal lead concentrations (in tertiles or log-transformed to the base of ten) and behavioral scores. ZIKV exposure was associated with a significantly increased opposition score of 0.8 [0.3; 1.2] and physical aggression of 0.5 [0.7; 0.9], as well as a higher hyperactivity score but the p -value was not reaching the statistical significance level. **Table 6** showed a significant interaction between ZIKV exposure and lead exposure for hyperactivity and inattention scores: a monotonous increase in both scores was observed in ZIKV (+) women, while a non-monotonic relationship was observed in ZIKV women (-). Results are presented in **Figures 6 and 7**, (p -interaction=0.02 and 0.10 respectively with additive scale). **Supplementary Table 3** showed a significant interaction between infant sex and maternal lead concentrations for physical aggression. No evidence of interaction between ZIKV exposure or infant sex and lead exposure was observed for the other behavioral scores (**Table 6**).

After excluding from the analysis those newborns with microcephaly (6%), the results were very similar in terms of the association with ASQ scores, a trend persisting between total, fine motor and problem-solving scores with lead in tertiles, and the association between ZIKV exposure and fine motor score (**Supplementary Table 4**). The association of ZIKV exposure with the hyperactivity score was reinforced, becoming statistically significant ($p=0.04$), while the association with the aggression score became non-significant (**Supplementary Table 5**). The interaction between ZIKV and lead exposure was no longer significant in the ASQ personal-social score (**Supplementary Table 6**). The other associations were similar (**Supplementary Tables 5 and 6**). When excluding infants with a probable in utero ZIKV exposure from the analysis (keeping only infants with a confirmed exposure classification), the results were fairly similar (**Supplementary Tables 7 to 9**). The fine motor subscale had stronger association for all analyses (lead in tertiles, log-transformed or ZIKV model) (**Supplementary Table 7**). Using complete case data, or excluding infants with medical conditions that could lead to development impairment, did not change the results (data not shown).

Discussion

In this birth cohort, set up in Guadeloupe during the 2016 ZIKV outbreaks, we found associations between prenatal lead exposure and neurodevelopmental delays at 18 months, with lower scores in total ASQ, fine motor and problem-solving domains. These associations appear to be boy-specific (total ASQ, fine motor, and personal and social scores). No associations were observed with infant behavior.

Decreases in ASQ problem-solving scores were strongest among the ASQ subscales and similar in boys and girls, and some of these associations were modified by ZIKV infection.

Our results corroborate those reported in many studies conducted among older children in terms of poorer cognitive development (including lower IQ) among children exposed prenatally to lead levels below 5 µg/dL (Desrochers-Couture et al. 2018; Fruh et al. 2019; Jedrychowski et al. 2009; Polanska et al. 2018; Tatsuta et al. 2020). Several of these studies observed these associations, as we did, in boys only (Desrochers-Couture et al. 2018; Polanska et al. 2018; Tatsuta et al. 2020). Alterations in behavior, such as attention deficit or hyperactivity, have been reported in association with prenatal exposure to lead at low levels (Neugebauer et al. 2015; Sioen et al. 2013) but were not observed here.

In our study, ZIKV infection during pregnancy was associated with a lower fine motor ASQ score, and higher scores for hyperactivity, opposition and aggressive behaviors at 18 months. When children with microcephaly at birth were excluded from the analysis, associations with ZIKV infection remained unchanged in terms of fine motor and opposition scores, reached significance for hyperactivity (from $p=0.08$ to 0.04), and became not significant with aggressive behavior (from $p=0.02$ to 0.07), suggesting that part of this association may be mediated by microcephaly. In a previous analysis of a partly overlapping cohort in the French West Indies and French Guiana that excluded babies with microcephaly at birth and also used the ASQ questionnaire – though completed by the mother when infants reached 24 months of age, “there would not appear to be an impact on longer term neurodevelopment outcomes attributable to in utero ZIKV exposure which may manifest after birth up to 24 months of age” (Grant et al. 2021). Other cohorts following normocephalic children potentially exposed to ZIKV infection in utero, reported decreases in neurodevelopmental outcomes trajectories up to age 18 months (Mulkey et al. 2020). A slower rate of communication skills development was observed over a 24-month period (Key et al. 2021), as well as loss of visual acuity and contrast sensitivity without evidence of losses in cognitive, motor or language development or head growth up to 30 months (Blackmon et al. 2022).

A few associations between neurodevelopment and prenatal lead exposure did appear to be modified by ZIKV infection, namely the ASQ personal-social score and behavior scores (hyperactivity, inattention). However, the interactions between prenatal lead exposure and ZIKV infection on ASQ personal-social and inattention scores were no longer significant when children with microcephaly were excluded. The interaction of lead and ZIKV observed on hyperactivity scores appeared to be driven mainly by differences in scores in those children highly exposed to lead (T3): in this T3 group, children of ZIKV (+) women showed the highest hyperactivity scores overall, compared to the less exposed children (T1-T2). However, children of ZIKV (-) women (expected to represent a control population) showed a decrease in hyperactivity score compared to the less exposed children; this is counterintuitive given the previous associations found between hyperactivity and prenatal lead exposure (Donzelli et al. 2019; He et al. 2019; Neugebauer et al. 2015). Lower fine motor scores were observed in association both with lead exposure and ZIKV infection, possibly a result of shared mechanism (altered migration, proliferation and differentiation of neural stem cells) (Gagnon-Chauvin et al. 2020, Wen et al. 2017, Huang and Schneider 2004) but there is only weak evidence for the presence of an interaction i.e. if any, effects of ZIKA infection and lead are additive not more.

Strengths

Our study was based on a relatively large population of mother-child pairs with well-characterized ZIKV-infection status during pregnancy (including an attempt to classify most of the population, with varying degrees of confidence, reducing the risk of selection bias). A number of confounders and co-exposures were collected at various phases of the study and expected associations with outcomes were present. Finally, a number of sensitivity analyses were conducted.

Limitations

Despite a careful ZIKV-exposure-classification process during the pregnancy, the exact period of infection during pregnancy could not be determined. Both animal (Block et al. 2020; Nguyen et al. 2017) and human (Brasil et al. 2016) studies have suggested that the most severe cases of CZS (e.g. microcephalia) were most likely to be due to infection early in pregnancy. Sensitivity analyses excluding microcephalic children at birth were conducted, but other features of CZS such as abnormal fetal brain findings on ultrasonography or magnetic resonance imaging were not excluded. Maternal blood lead level at delivery was used to characterize child exposure to lead. A number of cohort studies have shown that blood markers of lead environmental exposure are relatively stable during pregnancy (Arbuckle et al., 2016 ; Ettinger et al., 2004) and we consider that one single measurement of blood lead concentration at delivery is a reliable measure of prenatal exposure, and a correlate of breast milk contamination. Within this period of chronic exposure covering both in utero and early life exposure, no specific window of vulnerability can therefore be identified based on exposure measurement. A great many statistical tests were performed (including interactions with ZIKV exposure and with sex), and these may have resulted in some chance findings. For this reason, the plausibility of positive results was discussed in light of sensitivity analyses and previous knowledge. The limited size of the population may however have restrained our ability to identify interactions.

Conclusions

Our study confirms previous findings of associations between perinatal exposure to lead at low levels and adverse neurodevelopmental outcomes during infancy, and adds to evidence of a particular vulnerability among boys. It also suggests associations between ZIKV infection during pregnancy and subtle adverse effects on fine motor function, oppositional and hyperactive behaviors. These associations need to be confirmed at a later age and in other populations. Though there is no clear, consistent evidence of interaction between ZIKV infection and lead exposure, we are reporting consistent results supporting the hypothesis that both prenatal lead exposure and ZIKV infection during pregnancy are related to poorer fine motor function during infancy. Lower performance on fine motor function observed in association with ZIKV infection is comparable to what is

observed in the third tertile of lead concentrations (>1.3 µg/dL maternal blood), amounting to a loss of 12% in terms of fine motor function when both exposures were present (14% of the population).

References

- Adams L, Bello-Pagan M, Lozier M, Ryff KR, Espinet C, Torres J, et al. 2016. Update: Ongoing zika virus transmission - puerto rico, november 1, 2015-july 7, 2016. *MMWR Morb Mortal Wkly Rep* 65:774-779.
- Ahun MN, Consoli A, Pingault JB, Falissard B, Battaglia M, Boivin M, et al. 2018. Maternal depression symptoms and internalising problems in the offspring: The role of maternal and family factors. *Eur Child Adolesc Psychiatry* 27:921-932.
- Arbuckle TE, Liang CL, Morisset AS, Fisher M, Weiler H, Cirtiu CM, Legrand M, Davis K, Ettinger AS, Fraser WD; MIREC Study Group. 2016. Maternal and fetal exposure to cadmium, lead, manganese and mercury: The MIREC study. *Chemosphere*. 2016 Nov;163:270-282. doi: 10.1016/j.chemosphere.2016.08.023. Epub 2016 Aug 16. PMID: 27540762
- Bauer JA, Devick KL, Bobb JF, Coull BA, Bellinger D, Benedetti C, et al. 2020. Associations of a metal mixture measured in multiple biomarkers with iq: Evidence from italian adolescents living near ferroalloy industry. *Environ Health Perspect* 128:97002.
- Blackmon K, Evans R, Fernandes M, Landon B, Noel T, Macpherson C, et al. 2022. Neurodevelopment in normocephalic children with and without prenatal zika virus exposure. *Arch Dis Child* 107:244-250.
- Block LN, Aliota MT, Friedrich TC, Schotzko ML, Mean KD, Wiepz GJ, et al. 2020. Embryotoxic impact of zika virus in a rhesus macaque in vitro implantation model. *Biol Reprod* 102:806-816.
- Brasil P, Pereira JP, Jr., Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, et al. 2016. Zika virus infection in pregnant women in rio de janeiro. *N Engl J Med* 375:2321-2334.
- COFRAC. 2019. Cofrac accreditation in human health for the leres/ehesp laboratory Rennes.
- Crump KS, Van Landingham C, Bowers TS, Cahoy D, Chandalia JK. 2013. A statistical reevaluation of the data used in the lanphear et al. (2005) pooled-analysis that related low levels of blood lead to intellectual deficits in children. *Crit Rev Toxicol* 43:785-799.
- Desrochers-Couture M, Oulhote Y, Arbuckle TE, Fraser WD, Seguin JR, Ouellet E, et al. 2018. Prenatal, concurrent, and sex-specific associations between blood lead concentrations and iq in preschool canadian children. *Environ Int* 121:1235-1242.
- Donzelli G, Carducci A, Llopis-Gonzalez A, Verani M, Llopis-Morales A, Cioni L, et al. 2019. The association between lead and attention-deficit/hyperactivity disorder: A systematic review. *Int J Environ Res Public Health* 16.
- Ettinger AS, Téllez-Rojo MM, Amarasiriwardena C, Bellinger D, Peterson K, Schwartz J, Hu H, Hernández-Avila M. 2004. Effect of breast milk lead on infant blood lead levels at 1 month of age. *Environ Health Perspect*. 112(14):1381-5. doi: 10.1289/ehp.6616
- Freire C, Amaya E, Gil F, Fernandez MF, Murcia M, Llop S, et al. 2018. Prenatal co-exposure to neurotoxic metals and neurodevelopment in preschool children: The environment and childhood (inma) project. *Sci Total Environ* 621:340-351.
- Froehlich TE, Lanphear BP, Auinger P, Hornung R, Epstein JN, Braun J, et al. 2009. Association of tobacco and lead exposures with attention-deficit/hyperactivity disorder. *Pediatrics* 124:e1054-1063.
- Fruh V, Rifas-Shiman SL, Amarasiriwardena C, Cardenas A, Bellinger DC, Wise LA, et al. 2019. Prenatal lead exposure and childhood executive function and behavioral difficulties in project viva. *Neurotoxicology* 75:105-115.
- Gagnon-Chauvin A, Bastien K, Saint-Amour D. 2020. Environmental toxic agents: The impact of heavy metals and organochlorides on brain development. *Handb Clin Neurol* 173:423-442.

- Galera C, Cote SM, Bouvard MP, Pingault JB, Melchior M, Michel G, et al. 2011. Early risk factors for hyperactivity-impulsivity and inattention trajectories from age 17 months to 8 years. *Arch Gen Psychiatry* 68:1267-1275.
- Grant R, Flechelles O, Tressieres B, Dialo M, Elenga N, Mediamolle N, et al. 2021. In utero zika virus exposure and neurodevelopment at 24 months in toddlers normocephalic at birth: A cohort study. *BMC Med* 19:12.
- Guo J, Wu C, Zhang J, Qi X, Lv S, Jiang S, et al. 2020. Prenatal exposure to mixture of heavy metals, pesticides and phenols and iq in children at 7 years of age: The smbcs study. *Environ Int* 139:105692.
- HAS. 2016. Détection du virus zika par rt-pcr dans le sang et les urines – argumentaire:Haute Autorité de Santé.
- He J, Ning H, Huang R. 2019. Low blood lead levels and attention-deficit hyperactivity disorder in children: A systematic review and meta-analysis. *Environ Sci Pollut Res Int* 26:17875-17884.
- Hoen B, Schaub B, Funk AL, Ardillon V, Boullard M, Cabie A, et al. 2018. Pregnancy outcomes after zikv infection in french territories in the americas. *N Engl J Med* 378:985-994.
- Huang F, Schneider JS. 2004. Effects of lead exposure on proliferation and differentiation of neural stem cells derived from different regions of embryonic rat brain. *Neurotoxicology* 25:1001-1012.
- Huijbregts SC, Seguin JR, Zoccolillo M, Boivin M, Tremblay RE. 2007. Associations of maternal prenatal smoking with early childhood physical aggression, hyperactivity-impulsivity, and their co-occurrence. *J Abnorm Child Psychol* 35:203-215.
- Huijbregts SC, Seguin JR, Zoccolillo M, Boivin M, Tremblay RE. 2008. Maternal prenatal smoking, parental antisocial behavior, and early childhood physical aggression. *Dev Psychopathol* 20:437-453.
- Jedrychowski W, Perera FP, Jankowski J, Mrozek-Budzyn D, Mroz E, Flak E, et al. 2009. Very low prenatal exposure to lead and mental development of children in infancy and early childhood: Krakow prospective cohort study. *Neuroepidemiology* 32:270-278.
- Jin Y, Hein MJ, Deddens JA, Hines CJ. 2011. Analysis of lognormally distributed exposure data with repeated measures and values below the limit of detection using sas. *Ann Occup Hyg* 55:97-112.
- Key AP, Negrini S, Caldas C, Teixeira SR, Anastasio ART, Cavalcante J, et al. 2021. A prospective study of neurodevelopmental trends between 3 and 24 months in normocephalic infants with prenatal zika virus exposure: Evidence of emerging communication delays in the natzig cohort. *Early Hum Dev* 163:105470.
- Krauer F, Riesen M, Reveiz L, Oladapo OT, Martinez-Vega R, Porgo TV, et al. 2017. Zika virus infection as a cause of congenital brain abnormalities and guillain-barre syndrome: Systematic review. *PLoS Med* 14:e1002203.
- Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, et al. 2005. Low-level environmental lead exposure and children's intellectual function: An international pooled analysis. *Environ Health Perspect* 113:894-899.
- Lazarevic N, Barnett AG, Sly PD, Knibbs LD. 2019. Statistical methodology in studies of prenatal exposure to mixtures of endocrine-disrupting chemicals: A review of existing approaches and new alternatives. *Environ Health Perspect* 127:26001.
- McFarland MJ, Hauer ME, Reuben A. 2022. Half of us population exposed to adverse lead levels in early childhood. *Proc Natl Acad Sci U S A* 119:e2118631119.
- Melo AS, Aguiar RS, Amorim MM, Arruda MB, Melo FO, Ribeiro ST, et al. 2016. Congenital zika virus infection: Beyond neonatal microcephaly. *JAMA Neurol* 73:1407-1416.
- Mulkey SB, Arroyave-Wessel M, Peyton C, Bulas DI, Fourzali Y, Jiang J, et al. 2020. Neurodevelopmental abnormalities in children with in utero zika virus exposure without congenital zika syndrome. *JAMA Pediatr* 174:269-276.
- Neugebauer J, Wittsiepe J, Kasper-Sonnenberg M, Schoneck N, Scholmerich A, Wilhelm M. 2015. The influence of low level pre- and perinatal exposure to pcdd/fs, pcbs, and lead on attention performance and attention-related behavior among german school-aged children: Results from the duisburg birth cohort study. *Int J Hyg Environ Health* 218:153-162.
- Nguyen SM, Antony KM, Dudley DM, Kohn S, Simmons HA, Wolfe B, et al. 2017. Highly efficient maternal-fetal zika virus transmission in pregnant rhesus macaques. *PLoS Pathog* 13:e1006378.

- Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM. 2016. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: Tip of the iceberg? *Ultrasound Obstet Gynecol* 47:6-7.
- Pele F, Muckle G, Costet N, Garlantezec R, Monfort C, Multigner L, et al. 2013. Occupational solvent exposure during pregnancy and child behaviour at age 2. *Occup Environ Med* 70:114-119.
- Polanska K, Hanke W, Pawlas N, Wesolowska E, Jankowska A, Jagodic M, et al. 2018. Sex-dependent impact of low-level lead exposure during prenatal period on child psychomotor functions. *Int J Environ Res Public Health* 15.
- Pomar L, Musso D, Malinger G, Vouga M, Panchaud A, Baud D. 2019. Zika virus during pregnancy: From maternal exposure to congenital zika virus syndrome. *Prenat Diagn* 39:420-430.
- Radloff SL. 1977. The ces-d scale: A self-report depression scale for research in the general population. *Applied Psychological Measurement*:385-401.
- Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. 2016. Zika virus and birth defects--reviewing the evidence for causality. *N Engl J Med* 374:1981-1987.
- Sioen I, Den Hond E, Nelen V, Van de Mierop E, Croes K, Van Larebeke N, et al. 2013. Prenatal exposure to environmental contaminants and behavioural problems at age 7-8years. *Environ Int* 59:225-231.
- SpF. 2017. Le virus zika aux antilles et en guyane. *Bulletin de veille sanitaire:Santé Publique France*.
- Squires J, Bricker D. 2009. *Ages & stages questionnaires (asq-3): A parent-completed child-monitoring system*. Stanford:Paul Brookes Publishing Company.
- Tamayo YOM, Tellez-Rojo MM, Trejo-Valdivia B, Schnaas L, Osorio-Valencia E, Coull B, et al. 2017. Maternal stress modifies the effect of exposure to lead during pregnancy and 24-month old children's neurodevelopment. *Environ Int* 98:191-197.
- Tatsuta N, Nakai K, Kasanuma Y, Iwai-Shimada M, Sakamoto M, Murata K, et al. 2020. Prenatal and postnatal lead exposures and intellectual development among 12-year-old japanese children. *Environ Res* 189:109844.
- Taylor CM, Kordas K, Golding J, Emond AM. 2017. Effects of low-level prenatal lead exposure on child iq at 4 and 8 years in a uk birth cohort study. *Neurotoxicology* 62:162-169.
- Valentine G, Marquez L, Pammi M. 2016. Zika virus-associated microcephaly and eye lesions in the newborn. *J Pediatric Infect Dis Soc* 5:323-328.
- Valeri L, Mazumdar MM, Bobb JF, Claus Henn B, Rodrigues E, Sharif OIA, et al. 2017. The joint effect of prenatal exposure to metal mixtures on neurodevelopmental outcomes at 20-40 months of age: Evidence from rural bangladesh. *Environ Health Perspect* 125:067015.
- van der Linden V, Pessoa A, Dobyns W, Barkovich AJ, Junior HV, Filho EL, et al. 2016. Description of 13 infants born during october 2015-january 2016 with congenital zika virus infection without microcephaly at birth - brazil. *MMWR Morb Mortal Wkly Rep* 65:1343-1348.
- Villar J, Papageorghiou AT, Pang R, Ohuma EO, Cheikh Ismail L, Barros FC, et al. 2014. The likeness of fetal growth and newborn size across non-isolated populations in the intergrowth-21st project: The fetal growth longitudinal study and newborn cross-sectional study. *Lancet Diabetes Endocrinol* 2:781-792.
- Vuong AM, Xie C, Jandarov R, Dietrich KN, Zhang H, Sjodin A, et al. 2020. Prenatal exposure to a mixture of persistent organic pollutants (pops) and child reading skills at school age. *Int J Hyg Environ Health* 228:113527.
- Wen Z, Song H, Ming GL. 2017. How does zika virus cause microcephaly? *Genes Dev* 31:849-861.

Figure 1: Flow chart

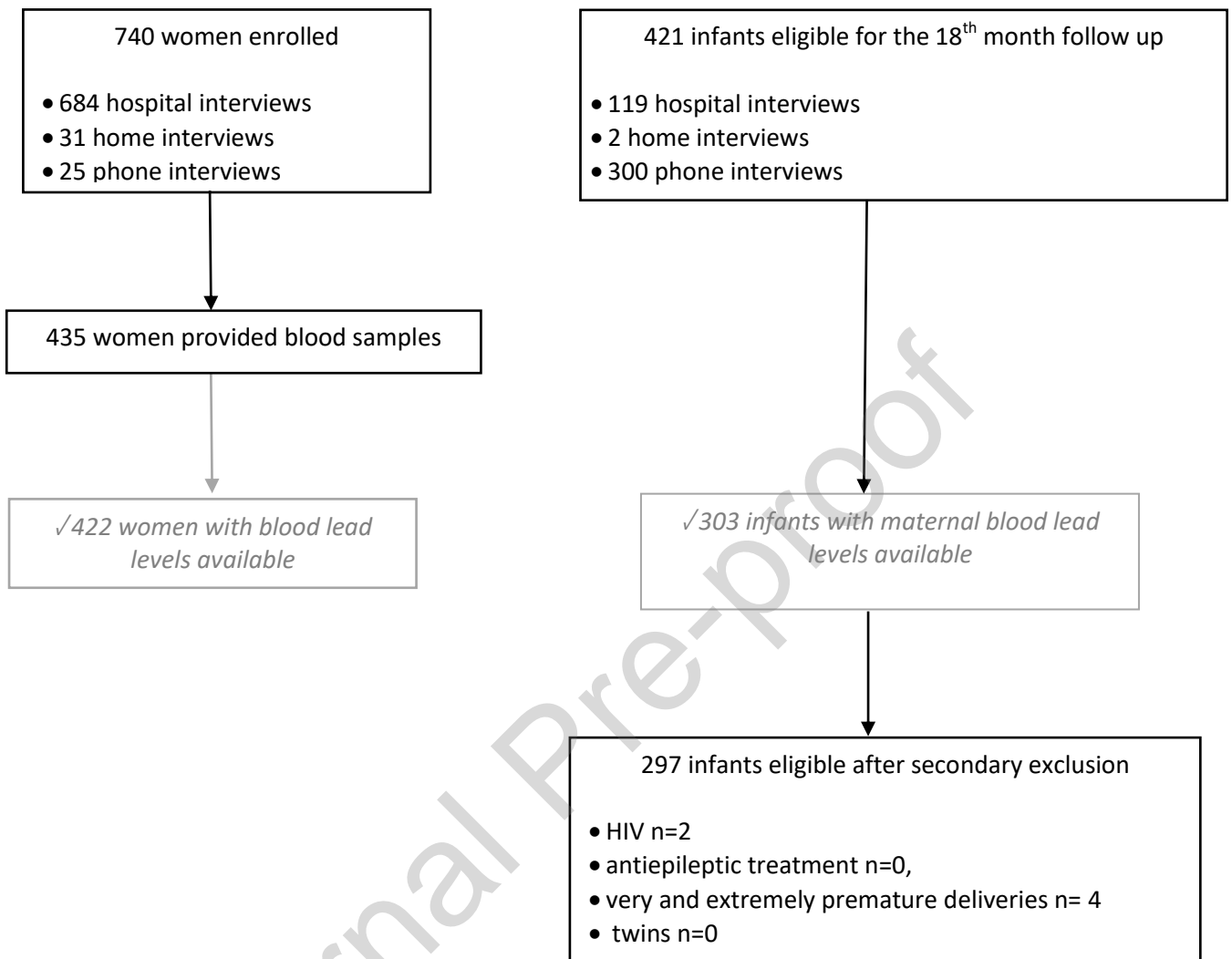


Table 1: Population characteristics (n=297) and determinants of ZIKV infection and blood lead levels at 18-month follow-up

	18-month follow-up N=297		Blood lead level (µ/L)		ZIKV		p*
	M V	% (N) or mean (±SD) [min Q1 Med Q3 max]	corr or mean [IC 95%]	p*	Yes (n=127) % (N) or mean (±SD)	No (n=170) % (N) or mean (±SD)	
Maternal characteristics							
Age at delivery (years)	0	30.5 (±6.31)	0.14	0.02	30.0 (±6.5)	31.5 (±6.3)	0.02
BMI (kg/m ²)	4	26.2 (±6.3)	0.16	0.00	26.9 (±6.8)	25.9 (±5.9)	0.1
Obesity	4						4
Underweight		5.7% (17)	12.3 [7.6; 17.1]	0.13	4.0% (5)	7.1% (12)	0.71
Normal		42.4% (126)	12.4 [10.6; 14.1]		43.6% (54)	42.6% (72)	
Overweight		30.0% (89)	13.6 [11.6; 15.7]		30.6% (38)	27.8% (47)	
Obese		21.9% (65)	15.9 [13.5; 18.4]		21.8% (27)	22.5% (38)	
Parity	1						
Primiparous		36.7% (109)	13.0 [10.7; 15.3]	0.50	41.7% (53)	32.9% (56)	0.12
Multiparous		63.3% (188)	13.8 [12.6; 15.1]		58.3% (74)	67.1% (114)	
Education level	0						
≤12 years		37.7% (112)	15.7 [13.6; 17.9]	0.01	39.4% (50)	36.5% (62)	0.61
>12 years		62.3% (185)	12.2 [10.9; 13.5]		60.6% (77)	63.5% (108)	
Place of birth	0						
French West Indies		60.9% (181)	12.1 [10.7; 13.5]	<.001	63.8% (80)	58.8% (99)	0.15
Other West Indies		26.9% (80)	18.1 [16.0; 20.2]		28.3% (37)	25.9% (45)	
Metropolitan France, Europe and other		12.1% (36)	10.6 [7.4; 13.7]		7.9% (10)	15.3% (26)	
Tobacco use during pregnancy	0						
No		90.9% (270)	13.6 [12.3; 14.8]	0.70	89.0% (113)	92.4% (157)	0.32
Yes		9.1% (27)	13.0 [10.5; 15.6]		11.0% (14)	7.6% (13)	
Marital status	1						
With a partner		53.4% (158)	13.0 [11.7; 14.6]	0.39	48.8% (62)	57.1% (97)	0.08
Alone with family members		17.9% (53)	12.9 [10.2; 15.7]		23.6% (30)	13.5% (23)	
Alone		28.7% (85)	14.8 [12.6; 16.9]		27.6% (35)	29.4% (50)	
Household income							
<450€		8.8% (26)	18.4 [14.5; 22.2]	0.14	10.3% (13)	7.7% (13)	0.17
451-800€		9.1% (27)	13.7 [9.5; 17.5]		11.8% (15)	7.1% (12)	
801-1500€		35.7% (106)	13.2 [11.3; 15.1]		39.4% (50)	32.9% (56)	
1501-3000€		29.3% (87)	12.9 [10.8; 15.0]		25.2% (32)	32.3% (55)	
>3000€		17.2% (51)	12.8 [10.0; 15.5]		13.4% (17)	20.0% (34)	

Maternal self-reported depression symptoms at 18 months ^a	2							
No	4	75.1% (223)	13.5 [12.1; 14.8]	0.84	66.1% (84)	81.8% (139)	0.0	02
Yes		24.9% (74)	13.7 [11.7; 15.7]		33.9% (43)	18.2% (31)		
Maternal blood levels of metals (µg/L)								
Lead (µg/L)	0				14.0 (±11.5)	13.2 (±8.7)	0.5	3
% detected		100% (297)						
concentration		13.5 (±10.0) [2.2 - 8.5 - 11.0 - 15.4 - 111]						
tertiles [min p33 p66 max]		[2.2 - 9.2 - 13.3 - 111]						
Arsenic (µg/L)	7						0.8	7
% detected	3	75% (224)	0.04	0.52	2.8 (±4.2)	2.8 (±4.1)		
concentration		2.8 (±4.1) [0.3 - 1.0 - 1.6 - 2.8 - 36.8]						
Cadmium (µg/L)	8				<.001	0.4 (±0.2)	0.4 (±0.2)	0.1
% detected	5	71% (212)	0.33					1
concentration		0.4 (±0.2) [0.1 - 0.2 - 0.3 - 0.4 - 1.7]						
Copper (µg/L)	0		0.04	0.53	1590.6 (±239.8)	1563.7 (±280.0)	0.3	8
% detected		100% (297)						
concentration		1575 (±263) [912 - 1408 - 1556 - 1733 - 2859]						
Mercury (µg/L)	1						0.8	5
% detected	0	97% (287)	0.09	0.13	2.1 (±1.4)	2.1 (±1.5)		
concentration		2.1 (±1.4) [0.2 - 1.2 - 1.8 - 2.6 - 9.1]						
Zinc (µg/L)	0		0.21	0.00	5757.0 (±1026.3)	5975.2 (±1213.0)	0.0	9
% detected		100% (297)						
concentration		5882 (±1140) [2809 - 5097 - 5766 - 6598 - 11252]						
Selenium (µg/L)	0		0.20	0.00	107.8 (±26.8)	105.5 (±17.4)	0.3	8
% detected		100% (422)						
concentration		106 (±22) [61 - 93 - 105 - 117 - 273]						
Manganese (µg/L)	1		0.21	0.00	15.9 (±5.1)	16.3 (±6.5)	0.5	3
% detected		99% (296)						
concentration		16.2 (±5.9) [3.4 - 12.6 - 15.1 - 18.9 - 43.8]						
Infant characteristics								
Birth weight (g)	0	3155 (±497)	-0.01	0.79	3126 (±482)	3116 (±540)	0.8	5
Gestational age at delivery (weeks)	0	38.9 (±1.5)	0.04	0.44	38.9 (±1.6)	38.6 (±1.7)	0.1	5
Sex	0							
Male		48.8% (145)	13.3 [11.7; 14.9]	0.71	45.7% (58)	51.2% (87)	0.3	5
Female		51.2% (152)	13.7 [12.1; 15.4]		54.3% (69)	48.8% (83)		
Breastfeeding duration (month)	2	7.7 (±6.7)	-0.08	0.15	7.7 (±6.7)	7.3 (±6.7)	0.5	6
Maternal stimulation score	9	11.9 (±2.0)	-0.08	0.17	11.9 (±2.1)	12.0 (±1.9)	0.6	3

Corrected age at 18m assessment (month) ^b	0	17.4 (±0.7)	-0.10	0.07	17.4 (±0.6)	17.2 (±0.7)	0.0
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MV: missing values, Min: minimum, Q1: first quartile, Med: median, Q3: third quartile, Max: maximum, p: p-values.

Description data and bivariate analysis were realized on imputed data, *p-values obtained from Student t, Chi², Spearman correlation (corr) or Anova tests.

^aAbbreviated (10-item) Center for Epidemiologic Studies Depression Scale.

^bAge at 18-month assessment corrected for gestational age.

1€=1.07US\$

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Table 2: Descriptives for 18-month outcomes

	18-month follow-up N=297		
	MV	mean (\pm SD)	range
Prenatal characteristics			
Gestational age at delivery (weeks)	0	38.9 (\pm 1.7)	33-41
Head circumference (cm)	0	33.8 (\pm 1.8)	27-38
Birth weight (g)	0	3155 (\pm 497)	1800-4680
Neurodevelopmental scores			
ASQ total score	0	222.6 (\pm 40.0)	0-300
ASQ communication	0	40.5 (\pm 15.1)	0-60
ASQ gross motor	0	56.4 (\pm 8.5)	0-60
ASQ fine Motor	0	45.3 (\pm 13.0)	0-60
ASQ personal-social	0	43.9 (\pm 9.5)	0-60
ASQ problem solving	0	36.4 (\pm 14.1)	0-60
Hyperactivity	9	12.5 (\pm 2.7)	6-18
Inattention	9	5.2 (\pm 1.5)	3-9
Opposition	9	8.2 (\pm 1.8)	4-12
Physical aggression	9	5.3 (\pm 1.8)	3-9

MV: missing values ASQ: Ages and Stages Questionnaire.

Table 3: Multivariable analysis between blood lead levels, ZIKV exposure and ASQ scores at 18 months (n=297)

	ASQ total score			ASQ gross motor			ASQ fine motor			ASQ personal-social			ASQ problem solving			ASQ communication			
	β [95% CI]	p	tr end	OR [95% CI]	p	tr end	β [95% CI]	p	tr end	β [95% CI]	p	tr end	β [95% CI]	p	tr end	β [95% CI]	p	tr end	
Blood lead levels																			
T1	(ref)	0.06	3	(ref)	0.4	40	(ref)	0.6	2	(ref)	0.8	1	(ref)	1	0.01	(ref)	0.8	3	
T2	-9.9 [-19.7; 0.1]			1.2 [0.6; 2.3]			-2.8 [-6.1; 0.4]			-2.3 [-4.8; 0.3]			-4.0 [-7.6; -0.4]			0.2 [-3.8; 4.2]			
T3	-11.8 [-22.5; 1.0]			1.3 [0.7; 2.6]			-3.9 [-7.3; 0.4]			-0.6 [-3.4; 2.2]			-8.3 [-12.0; -4.6]			-0.2 [-4.6; 4.2]			
Blood lead levels (log 10)	-19.3 [-39.8; 1.4]	0.07	7	1.7 [0.5; 5.7]	0.4	2	-5.9 [-12.2; 0.5]	0.7	7	2.3 [-3.1; 7.7]	0.3	9	-14.6 [-21.7; 7.6]	0.001	1	-1.3 [-9.6; 7.1]	0.7	6	
ZIKV	-5.9 [-14.1; 2.4]	0.1	6	1.4 [0.8; 2.3]	0.2	7	-3.5 [-6.3; 0.8]	0.1	1	-0.5 [-2.6; 1.6]	0.6	4	-0.15 [-3.2; 2.9]	0.92		-1.4 [-4.8; 1.9]	0.4	0	

ZIKV: Zika virus, ASQ: Ages and Stages Questionnaire, β coefficient and 95%CI obtained from linear regression, OR: odds ratio and 95%CI obtained from logistic regression.

Tertiles of lead concentration ($\mu\text{g/L}$): T1 [2.2 - 9.2], T2 [9.2 - 13.3], T3[13.3 - 111].

ASQ total adjusted for sex, birth weight, parity, maternal pre-pregnancy BMI, ZIKV, maternal cadmium levels, breastfeeding duration, maternal depression and stimulation scores; for the ZIKV model additionally adjusted for lead tertiles and infant's age at assessment.

ASQ gross motor adjusted for sex, stimulation score, household income at follow-up, maternal selenium and mercury levels; for the ZIKV model additionally adjusted for marital status and lead tertiles.

ASQ fine motor score adjusted on sex, parity, maternal pre-pregnancy BMI, maternal tobacco use during pregnancy, marital status, depression symptoms, breastfeeding duration, stimulation score, ZIKV and maternal mercury level; for the ZIKV model additionally adjusted for lead tertiles.

ASQ personal-social adjusted for sex, gestational age at delivery, birth weight, maternal education, stimulation score, and maternal cadmium and mercury levels; for the ZIKV model adjusted for sex, maternal education, stimulation score and maternal cadmium levels.

ASQ problem solving adjusted for sex, parity, maternal pre-pregnancy BMI, marital status, household income at follow-up and stimulation score; for the ZIKV model additionally adjusted for lead tertiles.

ASQ communication adjusted for sex, maternal pre-pregnancy BMI, infant's age at assessment, maternal depression symptoms, household income at follow-up, stimulation score and maternal cadmium levels.

Table 4: Interaction analysis between blood lead levels, ZIKV exposure and ASQ scores at 18 months (n=297)

	Blood lead levels	ZIKV-	ZIKV+	p interaction
		β [95% CI] or OR [95% CI]		
ASQ total score	T1	(ref)	-4.4 [-18.6; 9.9]	0.48
	T2	-7.0[-20.0; 6.0]	-18.0 [-31.6; -4.4]	
	T3	-14.2 [-28.0; -0.5]	-13.1 [-27.7; 1.4]	
ASQ gross motor	T1	(ref)	0.8 [0.3; 2.1]	0.47
	T2	0.9 [0.4; 2.3]	1.2 [0.67; 2.21]	
	T3	0.9 [0.4; 2.2]	1.3 [0.8; 2.1]	
ASQ fine motor	T1	(ref)	-3.7[-8.4; 0.9]	0.48
	T2	-2.1 [-6.4; 2.2]	-7.4 [-12.0; -2.8]	
	T3	-5.0 [-9.3; -0.7]	-6.3 [-11.0; -1.5]	
ASQ personal-social	T1	(ref)	-0.8 [-4.5; 2.9]	0.05
	T2	-1.1 [-4.6; 2.3]	-4.4 [-7.9; -0.9]	
	T3	-2.3 [-5.9; 1.3]	0.8 [-2.9; 4.6]	
ASQ problem solving	T1	(ref)	-0.3 [-8.1; 6.4]	0.84
	T2	-3.6 [-8.46; 1.1]	-4.7 [-9.7; 0.3]	
	T3	-8.9 [-8.4; -4.1]	-7.8 [-13.1; -2.5]	
ASQ communication	T1	(ref)	-3.2 [-8.9; 2.6]	0.74
	T2	-0.6 [-5.9; 4.7]	-1.6 [-7.2; 3.8]	
	T3	-1.4 [-7.1; 4.2]	-1.5 [-7.5; 4.4]	

ZIKV: Zika virus, ASQ: Ages and Stages Questionnaire, β coefficient and 95%CI obtained from linear regression, OR: Odds ratio and 95%CI obtained from logistic regression for the ASQ gross motor model, p-value for interaction (additive or multiplicative scale).

Tertiles of lead concentration ($\mu\text{g/L}$): T1 [2.2 - 9.2], T2 [9.2 - 13.3], T3[13.3 - 111].

ASQ total adjusted for sex, birth weight, parity, maternal pre-pregnancy BMI, ZIKV, maternal cadmium levels, breastfeeding duration, maternal depression and stimulation scores.

ASQ gross motor adjusted for sex, stimulation score, household income at follow-up, maternal selenium and mercury levels.

ASQ fine motor score adjusted for sex, parity, maternal pre-pregnancy BMI, maternal tobacco use during pregnancy, marital status, depression symptoms, breastfeeding duration, stimulation score and maternal mercury levels.

ASQ personal-social adjusted for sex, birth weight, gestational age at delivery, maternal education, stimulation score and maternal cadmium and mercury levels.

ASQ problem solving adjusted for sex, parity, maternal pre-pregnancy BMI, marital status, household income at follow-up and stimulation score.

ASQ communication adjusted for sex, maternal pre-pregnancy BMI, infant's age at assessment, maternal depression symptoms, household income at follow-up, stimulation score and maternal cadmium levels.

Figure 2: Personal-social ASQ-score adjusted means according to ZIKV infection and blood lead levels (n=297) during pregnancy; Tertiles of lead concentration ($\mu\text{g/L}$): T1 [2.2 - 9.2], T2 [9.2 - 13.3], T3[13.3 - 111]; p-value of interaction (additive scale) = 0.05

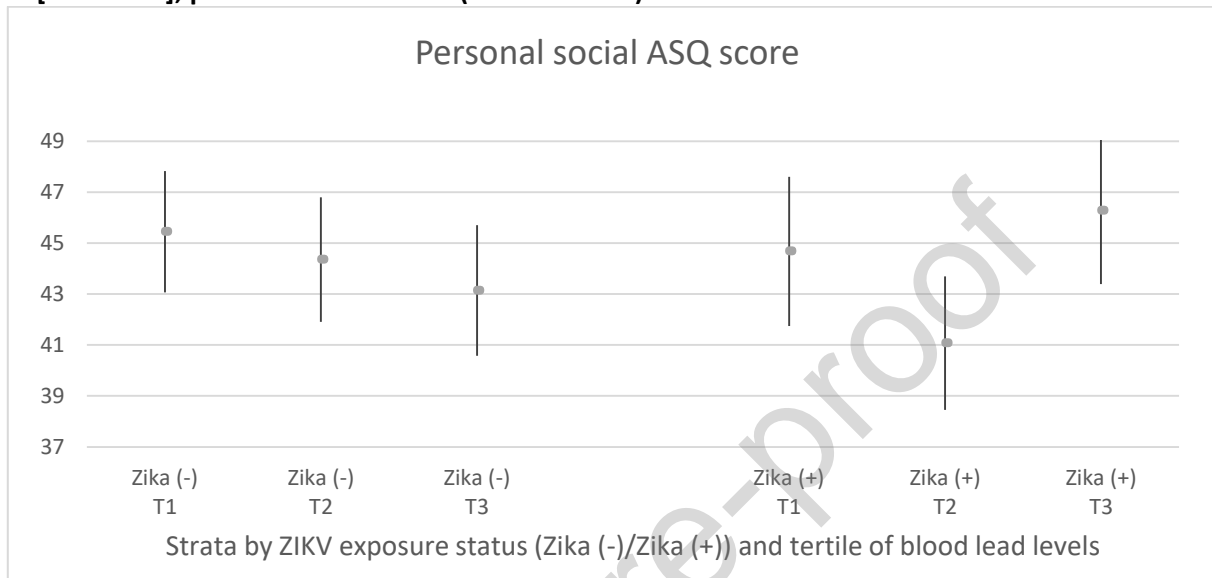


Table 5: Multivariable analysis between blood lead levels, ZIKV exposure and behavioral outcomes at 18-months (n=288)

	Hyperactivity			Opposition			Inattention			Physical aggression		
	β [95% CI]	p	ptrend	β [95% CI]	p	ptrend	β [95% CI]	p	ptrend	β [95% CI]	p	ptrend
Blood lead levels												
T1	(ref)	0.62	0.74	(ref)	0.44	0.72	(ref)	0.27	0.35	(ref)	0.89	0.68
T2	0.2 [-0.5; 1.0]			0.3 [-0.2; 0.8]			0.1 [-0.3; 0.5]			-0.1 [-0.6; 0.4]		
T3	-0.2 [-0.9; 0.6]			0.1 [-0.4; 0.6]			-0.2 [-0.6; 0.2]			-0.1 [-0.6; 0.4]		
Blood lead levels (log 10)												
ZIKV	-0.3 [-1.8; 1.3]	0.73		-0.0 [-0.9; 0.9]	0.94		-0.1 [-0.9; 0.7]	0.89		-0.2 [-1.1; 0.7]	0.70	
ZIKV	0.6 [-0.7; 1.2]	0.08		0.8 [0.3; 1.2]	0.0004		0.1 [-0.3; 0.4]	0.58		0.5 [0.7; 0.9]	0.02	

ZIKV: Zika virus, β coefficient and 95%CI obtained from linear regression.

Tertiles of lead concentration ($\mu\text{g/L}$): T1 [2.2 - 9.2], T2 [9.2 - 13.3], T3[13.3 - 111]

Hyperactivity score adjusted for sex, ZIKV and maternal depression and maternal cadmium levels; for the ZIKV model additionally adjusted for tobacco use during pregnancy.

Opposition score adjusted for sex, gestational age at delivery, parity, maternal education, household income, marital situation, maternal tobacco use during pregnancy, ZIKV and stimulation score.

Inattention score adjusted for sex, parity and maternal selenium levels; for the ZIKV model additionally adjusted for maternal education, pre-pregnancy BMI and stimulation score.

Physical aggression score adjusted for sex, parity, household income, maternal depression symptoms, stimulation score and ZIKV.

Table 6: Interaction analysis between blood lead levels, ZIKV exposure and behavioral scores at 18 months (n=288)

	Blood lead levels	ZIKV-	ZIKV+	p interaction
		β [95% CI]	β [95% CI]	
Hyperactivity	T1	(ref)	0.2 [-0.9; 1.3]	0.02
	T2	0.4 [-0.5; 1.4]	0.2 [-0.8; 1.3]	
	T3	-0.8 [-1.9; 0.2]	1.0 [-0.1; 2.1]	
Opposition	T1	(ref)	0.6 [-0.1; 1.3]	0.83
	T2	0.2 [-0.4; 0.8]	1.1 [0.4; 1.7]	
	T3	-0.1 [-0.7; 0.6]	0.8 [0.1; 1.5]	
Inattention	T1	(ref)	-0.1 [-0.7; 0.5]	0.10
	T2	0.2 [-0.3; 0.7]	-0.0 [-0.6; 0.5]	
	T3	-0.5 [-1.0; 0.1]	0.1 [-0.5; 0.7]	
Physical aggression	T1	(ref)	0.2 [-0.5; 0.9]	0.61
	T2	-0.2 [-0.9; 0.4]	0.3 [-0.4; 1.0]	
	T3	-0.3 [-0.9; 0.3]	0.4 [-0.3; 1.1]	

ZIKV: Zika virus, β coefficient and 95%CI obtained from linear regression, p-value for interaction additive or multiplicative scale.

Tertiles of lead concentration ($\mu\text{g/L}$): T1 [2.2 - 9.2], T2 [9.2 - 13.3], T3[13.3 - 111].

Hyperactivity score adjusted for sex, maternal depression and maternal cadmium levels.

Opposition score adjusted for sex, gestational age at delivery, parity, maternal education, household income, marital situation, maternal tobacco use during pregnancy and stimulation score.

Inattention score adjusted for sex, parity and maternal selenium levels.

Physical aggression score adjusted for sex, parity, household income, maternal depression symptoms, stimulation score and ZIKV.

Figure 6: Hyperactivity adjusted means scores according to ZIKV infection and blood lead levels (n=288) during pregnancy; Tertiles of lead concentration ($\mu\text{g/L}$): T1 [2.2 - 9.2], T2 [9.2 - 13.3], T3[13.3 - 111]; p-value of interaction (additive scale) = 0.02

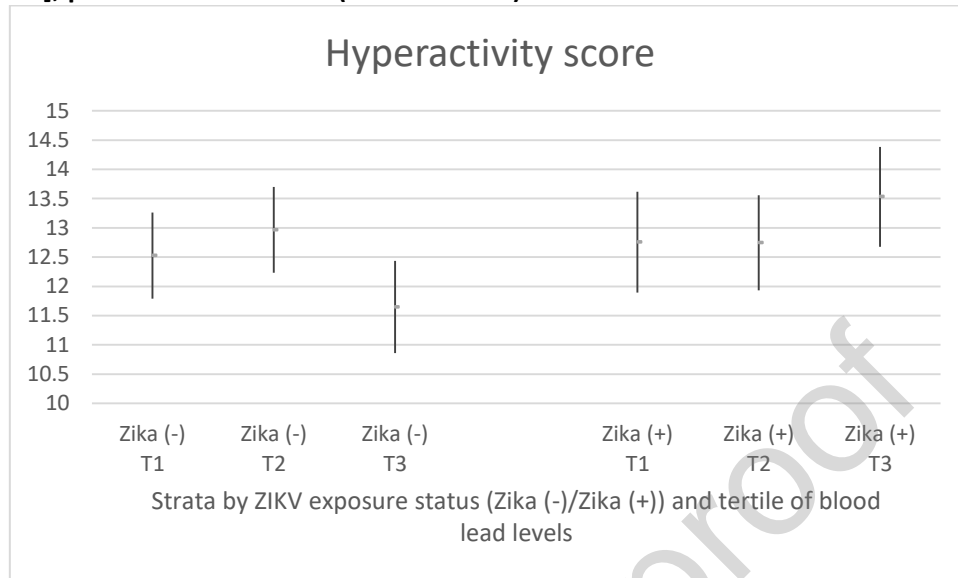
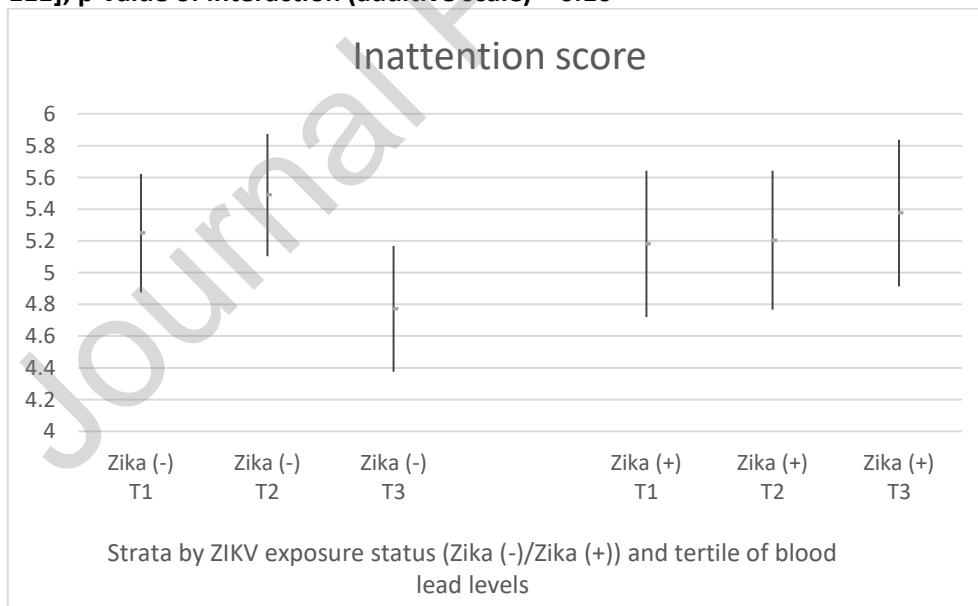


Figure 7: Inattention adjusted means score according to ZIKV infection and blood lead levels (n=288) during pregnancy; Tertiles of lead concentration ($\mu\text{g/L}$): T1 [2.2 - 9.2], T2 [9.2 - 13.3], T3[13.3 - 111]; p-value of interaction (additive scale) = 0.10



Authors' contributions

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Chemical analysis: BLB

Data analysis: MK, CC, SC

Writing – original draft: MK, GM, CC, SC

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All authors have read and approved the final manuscript.

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Declaration of interests

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

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Highlights (3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point):

- Knowledge on interaction between neurotoxic viruses and chemicals is scarce
- Guadeloupean pregnant women have been exposed to ZIKA virus and lead pollution
- Child neurodevelopmental and behavioral outcomes were assessed at 18 months
- Both Zika virus and lead affect fine motor domain, no interaction is evidenced
- Sex-specific neurodevelopment effects of low prenatal lead exposure are suggested

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