**Table 1**. Participant-level variables of interest

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Variable of interest** | **Var name in dataset** | **Definition** | **Done?** |
| Exposure | Maternal ZIKV infection | zikv\_preg  zikv\_test\_ev  zikv\_ga | Was pregnant woman diagnosed with ZIKV during this pregnancy using any criteria (clinical or laboratory diagnosis or self report) as defined by the study?  0=No; 1=Yes; 666=Not applicable; 888=Not reported by study; 999=Missing  Definitions Ricardo paper -> Robust, moderate, negative | No -> IgM DENV |
|  | Fetal or placental ZIKV infection | fet\_zikv | Fetal ZIKV infection? As defined by the study.  0=No; 1=Yes; 888=Not reported by study  Note: we will only use this variant of the variable. | Yes |
| Primary outcomes | Miscarriage (<20 weeks gestation) | miscarriage | Documented miscarriage: spontaneous loss of the product of the gestation <20 wks  0=No; 1=Yes; 555=Unknown; 888=Not measured by the study; 999=Missing | Yes |
|  |  | miscarriage\_ga | Gestational age of miscarriage (Weeks; miscarriage defined as spontaneous loss prior to 20 weeks is a miscarriage)  1-20 weeks; 666=Unknown; 888=Not measured by the study; 999=Missing |  |
|  |  | loss\_etiology (passive imputation)  if loss\_etiology=1 -> miscarriage=1  if loss\_etiology=0 -> miscarriage=0 | Cause of infant/fetus death  0=any live births (even if resulted in early/neo/perinatal death)  1=Intrauterine demise/Spontaneous abortion/miscarriage  2=Induced/Voluntary abortion  3=Fetal loss prior to labor (unknown whether spontaneous or voluntary)  4=Stillbirth or Intrapartum death (death during labor)  888=Not reported by study  999=Missing  Note: we cannot use 2 or 3 for passive imputation. |  |
|  | Fetal loss (≥20 weeks gestation) | Loss | Pregnancy loss (anything that is not a live birth is a pregnancy loss) | Yes |
|  |  | loss\_ga | Gestational age at pregnancy loss (Weeks)  1-45 weeks; 666=Unknown; 888=Not measured by the study; 999=Missing |  |
|  |  | loss\_etiology (passive imputation)  if loss\_etiology=2, 3 or 4 -> loss=1  if loss\_etiology=0 or 1 -> loss=0 | Cause of infant/fetus death  0=any live births (even if resulted in early/neo/perinatal death)  1=Intrauterine demise/Spontaneous abortion/miscarriage  2=Induced/Voluntary abortion  3=Fetal loss prior to labor (unknown whether spontaneous or voluntary)  4=Stillbirth or Intrapartum death (death during labor)  888=Not reported by study  999=Missing |  |
|  | Microcephaly (diagnosis: severe microcephaly, microcephaly, normocephaly, macrocephaly; Z-score) | fet\_micro | Prenatal Diagnosis of Microcephaly (Fetal microcephaly) | No (Johanna) |
|  |  | fet\_us\_micro\_tri1, fet\_us\_micro\_tri2, fet\_us\_micro\_tri3 | Microcephaly detected on ultrasound in 1st/2nd/3rd trimester  0=No; 1=Yes; 666=Not applicable; 888=Not reported by study; 999=Missing  Take the variable \_tri3, if missing use \_tri1 and \_tri2 for passive imputation. |  |
|  | CZS (diagnosis: confirmed, probable, unlikely) | ch\_czs (can also be defined based on other variables)  czs (WHO definition)   * Create based on the following:   zikv\_preg=1 OR fet\_zikv=1  AND  microcephaly=1 OR anyabnormatlity=1 | Diagnosis of congenital Zika syndrome (as measured by the study)  0=No; 1=Yes; 555=Unknown; 888=Not measured by the study; 999=Missing  WHO definition: Presence of confirmed maternal or fetal ZIKV infection AND presence of severe microcephaly OR presence of other malformations (eye, nose, ears etc.) | Yes |
| Secondary fetal outcomes | Induced abortion with microcephaly (diagnosis: confirmed, probable, unlikely) | No match |  | - |
|  | Early fetal death (20-27 weeks gestation) | efdeath -> will be computed after imputation based on loss and loss\_ga | Computed after imputation, based on loss and loss\_ga | - |
|  | Late fetal death (≥28 weeks gestation) | lfdeath -> will be computed after imputation based on loss and loss\_ga | Computed after imputation, based on loss and loss\_ga | - |
|  | Late fetal death (≥28 weeks gestation) with microcephaly | lfdeath\_micro -> will be computed after imputation based on loss and loss\_ga in combination with fet\_micro, fet\_us\_micro\_tri1, fet\_us\_micro\_tri2, fet\_us\_micro\_tri3 if 1 |  | - |
|  | Placental insufficiency (diagnosis: confirmed, probable, unlikely)‡ | No match |  | - |
|  | Intrauterine growth restriction | igr\_curr\_prg | Evidence of Intrauterine Growth Restriction (IGR)  0=No; 1=Yes; 555=Unknown; 888=Not measured by the study; 999=Missing | yes |
| Secondary infant outcomes | Postnatal microcephaly (diagnosis: severe microcephaly, microcephaly, normocephaly, macrocephaly; Z-score) | ch\_microcephaly | Level of microcephaly - as defined by the study.  0=Normocephaly; 1=Microcephaly; 2=Severe microcephaly; 888=Not reported by study; 999=Missing | Yes |
|  |  | ch\_microcephaly\_bin  Passive imputation  if ch\_microcephaly=1 or 2 -> ch\_microcephaly\_bin=1  if ch\_microcephaly=0 -> ch\_microcephaly\_bin=0 | Ever diagnosed with microcephaly? As defined by the study.  1=Yes; 0=No; 888=Not reported by study; 999=Missing |  |
|  |  | ch\_head\_circ\_birth, ch\_head\_circ\_1, ch\_head\_circ\_age\_1  -> Use these variables to compute microcephaly, using the intergrowth package |  |  |
|  | Gestational age at birth | birth\_ga | Gestational age in weeks at birth (live births) (note, value must be >=21 weeks)  555=Unknown; 888=Not measure by the study; 999=Missing | Yes |
|  | Birth weight (diagnosis: normal birth weight; low birth weight; very low birth weight; extremely low birth weight; Z-score) | ch\_weight | Birth weight in grams (<12 hours after delivery)  555555=Unknown; 888=Not measure by the study; 999=Missing; 666= missing?? | Yes |
|  | Craniofacial disproportion | ch\_craniofac\_abn\_bin | Presence of any other cranio-facial abnormalities (head abnormalities) - other than microcephaly  1=Yes; 0=No; 888=Not reported by study; 999=Missing | Yes |
|  | Neuroimaging abnormalities (intracranial calcification, lissencephaly, hydranencephaly, porencephaly, ventriculomegaly, posterior fossa abnormalities, cerebellar hypoplasia, corpus callosal and vermian dysgenesis; focal cortical dysplasia) | neuroabnormality   * We propose to create this based on the following:   fet\_us\_cns\_tri2 or fet\_us\_cns\_tri3 or ch\_hydrocephaly or ch\_corticalatrophy or ch\_calcifications or ch\_ventriculomegaly if 1  Also combine with the microcephaly variables specified above | Abnormal finding for central nervous system (anencephaly, microcephaly, spina bifida, encephalocele, hydrocephalus, holoproscencephaly, corticalatrophy, brain calcifications, ventriculomegaly)  0=No; 1=Yes | Yes |
|  | Postnatal intraventricular hemorrhage | No match |  | - |
|  | Any congenital abnormality on MRI or ultrasound | anyabnormality -> will be computed after imputation  We propose to construct this based on the following:  ch\_microcephaly\_bin,  ch\_microcephaly  ch\_craniofac\_abn\_bin,  neuroabnormality (see above),  ocularabnormality (see below),  contractures (see below),  nonneurologic (see below),  fet\_us\_micro\_tri1, fet\_us\_micro\_tri2, fet\_us\_micro\_tri3  if 1 | Abnormal finding for cranio-facial abnormalities, musculoskeletal system (club foot, Limb deficiency, Reduction deformity upper limbs, Reduction deformity upper limbs, hip dysplasia), cardiovascular system (ventricular septal defect without an associated genetic syndrome, transposition or totally anomalous pulmonary venous connection, Tetralogy of Fallot, functionally univentricular heart, hypoplastic left heart syndrome), gastrointestinal system (Gastroschisis, omphalocele, Diaphragmatic hernia, Atresia: choanal, esophageal, intestinal, biliary, rectal), oro-facial finding (cleft palate, cleft lip), eye-ear finding (Anophthalmia, microphthalmia, cataracts, anotia, microtia), genitourinary system (Hypospadias, Hermaphroditism, Phimosis, renal agenesis) | Yes |
|  | Motor abnormalities (hypotonia, hypertonia, hyperreflexia, spasticity, clonus, extrapyramidal symptoms)§ | No match |  | - |
|  | Seizures, epilepsy§ | No match |  | - |
|  | Ocular abnormalities (blindness, other)§ | ocularabnormality   * We propose to create this based on the following:   Fet\_us\_eyeear\_tri2, fet\_us\_eyeear\_tri3, if 1 | Abnormal eye-ear finding (Anophthalmia/microphthalmia, Cataracts, anotia, microtia) detected on 1st, 2nd or 3rd trimester ultrasound  0=No; 1=Yes | Yes |
|  | Congenital deafness or hearing loss§ | This variable will be combined with ocularabnormalities. |  | - |
|  | Congenital contractures (arthrogryposis, uni or bilateral clubfoot) | contractures  Created based on the following:  Fet\_us\_msk\_tri2, Fet\_us\_msk\_tri3, if 1 | Abnormal finding for musculoskeletal system (club foot, Limb deficiency, Reduction deformity upper limbs, Reduction deformity upper limbs, hip dysplasia) detected on 1st, 2nd or 3rd trimester ultrasound  0=No; 1=Yes | Yes |
|  | Other non-neurologic congenital abnormalities | nonneurologic  Propose to create based on the following:  fet\_us\_cardio\_tri2,  fet\_us\_gastro\_tri2,  fet\_us\_orofac\_tri2,  fet\_us\_genur\_tri2,  fet\_us\_cardio\_tri3,  fet\_us\_gastro\_tri3,  fet\_us\_orofac\_tri3,  fet\_us\_genur\_tri3, if equal to 1 |  | Yes |
| Secondary outcomes after infant period | Cortical auditory processing | No match |  | - |
|  | Neurodevelopment (expressive and receptive language, fine and gross motor skills, attention and executive function, memory and learning, socioemotional development, overall neurodevelopmental score) | No match |  | - |
|  | Vision (Cardiff test) | No match |  | - |
| Confounders | Demographic factors - age | age | Age of the mother in years. Continuous.  888=Not measured by the study; 999=Missing | Yes |
|  | Demographic factors - education | educ | Mother's highest level of education received  0= No education; 1=Primary school ; 2=Secondary school ; 3=Some college; 4=Bachelor's degree ; 5=Graduate or Professional degree ; 777=Other ; 888=Not reported by study ;999=Missing | Yes |
|  | Demographic factors – marital status | maritalstat | Mother's marital status  1=Single; 2=Married/Living as married/Cohabitating; 3=Divorced/Separated; 4=Widowed; 777=Other; 888=Not reported by study; 999=Missing | Yes |
|  | Demographic factors – racial / ethnic group | ethnicity | Maternal ethnicity as defined by the study  0=Caucasian descent; 1=African descent; 2=East Asian descent; 3=South Asian descent; 4=Indigenous descent; 5=Mixed; 777=Other; 888=Not reported by study; 999=Missing | Yes |
|  | Demographic factors - BMI | pre\_pregweight | Pre-pregnancy weight, in kg  888=Not measured by the study;  999=Missing | Yes |
|  |  | height | Height, in cm  888=Not measured by the study;  999=Missing |  |
|  | Socioeconomic factors | Ses | Maternal socioeconomic status identified as low, medium or high SES, or income  0=Low; 1=Medium; 2=High ; 777=Other ; 888=Not reported by study ; 999=Missing | Yes |
|  | Maternal smoking, illicit drug and alcohol use | tobacco  drugs\_bin  alcohol | Mother smokes tobacco during the current pregnancy  0=No; 1=Yes (Smoking currently, during current pregnancy); 2=Previous smoker (Before current pregnancy); 888=Not reported by study; 999=Missing (including unknown)  Current maternal (illicit) drug use or opioid substitution therapy (during pregnancy)  0=No; 1=Yes; 888=Not reported by study; 999=Missing  Maternal alcohol consumption during the current pregnancy  0=No; 1=Yes (any amount); 888=Not reported by study; 999=Missing (inlcuding unknonw) | Yes |
|  | Maternal prescription drug use -> this will be changed to teratogenic drug use | drug\_tera  ~~drugs\_prescr~~   * ~~We propose to create based on the following:~~   ~~med\_bin med\_anticonvuls\_bin, med\_fertil\_bin if 1~~  ~~med if 1-11 or 777~~  ~~Computed based on the following:~~  med\_anticonvuls\_bin if 1  med\_other if: tetracyclines, fluoroquinolones, antifungals, azoles, especially fluconazole, and warfarin | Indicate if the (pregnant) woman use any type of medications during the current pregnancy  0=none 1= painkiller 2=anticonvulsivant 3= anti-nausea 4=diuretic 5=anti-hypertensive 6= sleep medication 7=antiretrovirals or protease inhibitors (ARV/IP) 8= antibiotic 9= anti-depressive 10=immunosuppressants 11= inotropes 777=Other 555=Unknown 888=Not measured by the study 999=Missing | Yes |
|  | Maternal vaccination | vaccination   * Create based on the following:   vac\_rub,  vac\_vari,  vac\_yf if 1 | History of rubella, varicella or yellow fever vaccination at enrolment | Yes |
|  | Maternal experience of violence during pregnancy; infant or child exposure to intimate partner violence68 | No match |  | - |
|  | Workplace or environmental exposures to teratogenic substances (e.g. maternal exposure to lead, mercury) | No match |  | - |
| Effect measure modifiers | Genetic anomalies, metabolic disorders, perinatal brain injury (child) | gen\_anomalies:   * Create based on the following chromoabn\_dx and ch\_chromoabn, if 1-> more than 90% missing   Metabolic disorders: no match  Perinatal brain injury: no match | Chromosomal abnormalities diagnosed during this specific pregnancy  0=No (Normal); 1=Yes (Abnormal); 555=Unknown; 888=Not measured by the study; 999=Missing  Chromosomal abnormalities identified after delivery  0=No (Normal); 1=Yes (Abnormal); 555=Unknown; 888=Not measured by the study; 999=Missing | Yes |
|  | Gestational age, term at birth | birth\_ga (duplicate variable – also outcome) | Gestational age in weeks at birth (live births) (note, value must be >=21 weeks)  555=Unknown; 888=Not measure by the study; 999=Missing | Yes |
|  | Timing of infection during pregnancy | zikv\_ga | Gestational age at which women diagnosed with ZIKV, by EITHER ultrasound or LMP, in weeks. If both (ultrasound and LMP) information is avaiblable, priorotize ultrasound's GA information.  1-45 weeks;  666=Not Applicable (tested before current pregancy); 888=Not measured by the study; 999=Missing | Yes |
|  | Clinical/subclinical illness | No match |  | - |
|  | Viral genotype and load | Genotype: No match  Viral load: zikv\_pcr\_vl\_1 | Viral load (copies/µL) for a PCR for 1st ZIKV PCR  Continuous  666=Not applicable (no PCR done); 888=Not reported by study; 999=Missing | Yes |
|  | Concurrent or prior flavi- or alphavirus infection -> split into DENV and CHIKV | denv\_preg\_ever   * Create based on the following:   denv\_preg, denv\_ever if 1  chikv\_preg\_ever   * Create based on the following:   chikv\_preg, chikv\_ever if 1 | Concurrent or prior DENV  Concurrent or prior CHIKV | Yes |
|  | Maternal history of Yellow Fever (YF) or Japanese encephalitis (JE) vaccination | No match | Vaccination is already covered in variable vaccination | - |
|  | Maternal immunosuppressive conditions, disorders, comorbidities (e.g. chronic hypertension, diabetes), or pregnancy-related conditions (e.g. pre-eclampsia, gestational diabetes) -> split into two! | comorbid\_bin,  comorbid\_preg   * Create based on the following:   pregcomp\_bin, gestdiab, eclampsia, preeclampsia, if 1 | Presence of comorbidities (i.e: chronic/ pre-existent/ conditions) before the current pregnancy?  0=No; 1=Yes; 888=Not measured by the study; 999=Missing  Presence of complications during this specific pregnancy (including gestational diabetes, eclampsia, pre-eclampsia)  0=No; 1=Yes; 888=Not measured by the study; 999=Missing | Yes |
|  | Intrauterine exposure to STORCH pathogens | storch\_patho | Evidence of any (overall) STORCH pathogen infection during the current pregnancy?  0=No; 1=Yes; 888=Not measured by the study; 999=Missing | Yes |
|  | Maternal malnutrition | No match |  | - |
|  | Presence and severity of maternal and infant clinical symptoms -> consider separate variables | arb\_symp  fever  rash  arthralgia  headache  No match for infant clinical symptoms.  Not considered because too few data:  muscle\_pain  arthritis  vomiting  abd\_pain  bleed  fatigue  sorethroat | Any arbovirus-related symptoms during the current pregnancy  Fever present at any time point during the current pregnancy  Rash present at any time point during the current pregnancy  Joint pain/arthralgia present at any time point during the current pregnancy  Headache present at any time point during the current pregnancy  All:  0=No 1=Yes 888=Not reported by study 999=Missing | Yes |

CZS=congenital Zika syndrome, JE=Japanese encephalitis; STORCH=syphilis, toxoplasmosis, rubella, cytomegalovirus, and herpes; YF=yellow fever virus; ZIKV=Zika virus

\*Fetal ZIKV infection will be considered as both an exposure and an outcome; definition of fetal infection will be based on clinical and radiological criteria defined by an expert panel

†Both with and without microcephaly

‡As estimated by antenatal consequences of placental insufficiency, including fetal growth restriction, oligohydramnios, non-reassuring fetal heart rate tracing or small for gestational age at birth as markers of placental insufficiency.

§May also be detected after the infant period

\*\* As measured by the Bayley Scale;69 Ages and Stages;70 INTERGROWTH-21st Neurodevelopmental Assessment49

**Exposure workgroup meeting notes – 8 August 2022**

Attendees: Mabel Carabali, Ana Gorini da Veiga, Ernesto Marques, Deolinda Scalabrin, Ingrid Rabe, Janet Sayers, Ricardo Ximenes,

Discussion:

1. AD question regarding zikv\_preg variable: “We can try to construct this based on the individual test results. See the paper by Ricardo, I have tried to match the flow charts presented in the paper to variables in the dataset. Could you please check whether I did that correctly, and help me filling in the gaps?”

* Per Mabel: the zikv\_preg variable indicates whether the woman was diagnosed as having Zika virus infection, regardless of the criteria used. In reviewing the data, there were various combinations of laboratory and clinical criteria used. This variable was created to capture instances where the study reported that Zika was diagnosed but did not provide laboratory results. The stats team wants to impute the missing values of this variable using these test result variables. Given the complexity of diagnosis, a yes or no to this variable would be too simplistic to determine risk by level of evidence and timing of infection.
* The test results can be used to determine (1) very broadly “zikv\_preg” as yes/no depending on whether any markers of recent infection were detected namely, RNA detection on RT-PCR, IgM, IgG3 (not other general IgG), and PRNT seroconversion/fourfold increase in titre; and then (2) to determine the robustness of the evidence of recent infection (i.e., robust, moderate, and limited).
* The “limited evidence” category, for example, is borderline – could be positive or not; it will be important to delineate the importance of these types of results
* Matching the variables to the flowchart indicators is relevant to (2) above. The stratification into robust, moderate, and limited evidence of infection needs to be addressed in the analysis and the parameters in the algorithm (annotations to the pdf) appear to be correct.
* Clinical features in the absence of laboratory evidence is insufficient to diagnose patients as having ZIKV infection i.e., laboratory evidence may or may not be accompanied by symptoms (e.g., when infection is detection in asymptomatic persons). Thus, we should not use clinical only (in the absence of laboratory evidence) as “zikv\_preg” = yes.
* The timing of the laboratory test is very important and needs to be identified where possible for determination of risk by trimester of infection.
* However, for many cases, we will not have the temporality – in these cases there are follow up questions that could be used to determine the timing of infection.
* Post-pregnancy PCR could be viewed as an additional category – this may be the only evidence of infection if a woman was not tested during pregnancy but depending on how long after the pregnancy the specimen was taken, it may not indicate that infection occurred during pregnancy.
* In looking at the laboratory results, we still need to bear in mind that no laboratory tests are perfect; although PCR is more specific, false positives and negatives do occur depending on assay and laboratory proficiency and procedures.
* For the review of variables on the flowcharts (where Anneke had made annotations on the pdf)
  + Any IgG other than IgG3 should not be used as evidence of infection
  + “Do we have any post-pregnancy serologic tests?” – yes, there is a variable for puerperal serologic test results

1. AD question regarding fet\_zikv variable: “Do we also want to define this variable based on other variables? If so, how can we do it?”

* It is important to consider whether this is an exposure variable (i.e., evidence of maternal infection having occurred in pregnancy) or an outcome variable (i.e., evidence of infection in the fetus/neonate)
* There are difficulties in determining whether infection is fetal or maternal in many of the specimen types; if this is used as an exposure variable, i.e., evidence of maternal infection during pregnancy, it doesn’t matter. This is a different issue if this is to be used as an outcome variable, where there needs to be certainty of whether infection is confirmed from fetal vs maternal sources.
* This variable was created because there are numerous variables related to fetal testing and those could not be collapsed into this one. Refers to whether the study considered fetal Zika virus infection. Once again, this does require laboratory evidence of fetal infection (rather than just a report of yes/no without laboratory evidence of infection).
* There are numerous relevant variables (N=27) but whether the data are available or not is a different question