**OBJECTIVES OF THE IPD-MA**

1. Estimate the absolute and relative risks of fetal infection; miscarriage (<20 weeks gestation), fetal loss (≥ 20 weeks gestation), microcephaly, and other manifestations of CZS and later developmental delays for women who do and do not experience ZIKV infection during pregnancy.
2. Identify factors that modify women’s risk of adverse ZIKV-related fetal, infant, and child outcomes and infants’ risk of infection (e.g. gestational age at time of infection, clinical or subclinical illness, concurrent or prior arbovirus exposure, other congenital infections, and other posited effect measure modifiers).

Relevant variables

**Table 1**. Exposures

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| --- | --- |
| Exposure | Maternal ZIKV infection (diagnosis: confirmed, probable, unlikely; primary, secondary, naïve; viral load) |
|  | Fetal or placental ZIKV infection (diagnosis: confirmed, probable, unlikely; primary, secondary, naïve; viral load)\* |

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

**Table 2.** Outcomes

|  |  |  |
| --- | --- | --- |
|  | **Definition** | **Type** |
| Primary outcomes | Miscarriage (<20 weeks gestation) | Binary |
|  | Fetal loss (≥20 weeks gestation) | Binary |
|  | Microcephaly (diagnosis: severe microcephaly, microcephaly, normocephaly, macrocephaly; Z-score) | Ordinal / continuous |
|  | CZS (diagnosis: confirmed, probable, unlikely) | Ordinal |
| Secondary fetal outcomes† | Induced abortion with microcephaly (diagnosis: confirmed, probable, unlikely) | Ordinal |
|  | Early fetal death (20-27 weeks gestation) | Binary |
|  | Late fetal death (≥28 weeks gestation) | Binary |
|  | Late fetal death (≥28 weeks gestation) with microcephaly | Binary |
|  | Placental insufficiency (diagnosis: confirmed, probable, unlikely)‡ | Ordinal |
|  | Intrauterine growth restriction | Binary |
| Secondary infant outcomes† | Postnatal microcephaly (diagnosis: severe microcephaly, microcephaly, normocephaly, macrocephaly; Z-score) | Ordinal / continuous |
|  | Gestational age at birth | Continuous |
|  | Birth weight (diagnosis: normal birth weight; low birth weight; very low birth weight; extremely low birth weight; Z-score) | Ordinal / continuous |
|  | Craniofacial disproportion | Binary |
|  | Neuroimaging abnormalities (intracranial calcification, lissencephaly, hydranencephaly, porencephaly, ventriculomegaly, posterior fossa abnormalities, cerebellar hypoplasia, corpus callosal and vermian dysgenesis; focal cortical dysplasia) | Categorical / binary |
|  | Postnatal intraventricular hemorrhage | Binary |
|  | Motor abnormalities (hypotonia, hypertonia, hyperreflexia, spasticity, clonus, extrapyramidal symptoms)§ | Categorical / binary |
|  | Seizures, epilepsy§ | Binary |
|  | Ocular abnormalities (blindness, other)§ | Categorical / binary |
|  | Congenital deafness or hearing loss§ | Binary |
|  | Congenital contractures (arthrogryposis, uni or bilateral clubfoot) | Categorical / binary |
|  | Other non-neurologic congenital abnormalities | Binary |
| Secondary outcomes detected after the infant period | Cortical auditory processing | Survival |
|  | Neurodevelopment (expressive and receptive language, fine and gross motor skills, attention and executive function, memory and learning, socioemotional development, overall neurodevelopmental score) | Survival |
|  | Vision (Cardiff test) | Survival |

CZS=congenital Zika syndrome

†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

**Table 3**. Covariates

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| --- | --- |
| Posited confounders | Demographic factors (age, education, marital status, racial/ethnic group; BMI) |
|  | Socioeconomic factors |
|  | Maternal smoking, illicit drug and alcohol use |
|  | Maternal prescription drug use, vaccination |
|  | Maternal experience of violence during pregnancy; infant or child exposure to intimate partner violence15 |
|  | Workplace or environmental exposures to teratogenic substances (e.g. maternal exposure to lead, mercury) |
| Potential effect measure modifiers | Genetic anomalies, metabolic disorders, perinatal brain injury |
|  | Gestational age, term at birth |
|  | Timing of infection during pregnancy |
|  | Clinical/subclinical illness |
|  | Viral genotype and load |
|  | Concurrent or prior flavi- or alphavirus infection |
|  | Maternal history of YF or JE vaccination |
|  | Maternal immunosuppressive conditions, disorders, comorbidities (e.g. chronic hypertension, diabetes), or pregnancy-related conditions (e.g. pre-eclampsia, gestational diabetes) |
|  | Intrauterine exposure to STORCH pathogens |
|  | Maternal malnutrition |
|  | Presence and severity of maternal and infant clinical symptoms |

JE=Japanese encephalitis; STORCH=syphilis, toxoplasmosis, rubella, cytomegalovirus, and herpes; YF=yellow fever virus

General considerations

* Only outcomes of sufficient data quality (definitions, missings)
* Try to harmonize definitions as much as possible based on original (e.g. continuous) variables
* Bonferroni correction

Objective 1

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| --- | --- | --- |
| **Task** | **Status** | **Done?** |
| Data cleaning |  |  |
| Microcephaly: uniform definition results in many macrocephaly cases. Ask whether this is to be expected. Cross-tab with microcephaly as defined by the study (Johanna). |  |  |
| CZS. Prevalence is high, but definition is checked and it is correct (Johanna will make one change)  Definition: microcephaly OR other comorbidity |  |  |
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| ***Descriptives*** | | |
| Histograms of continuous variables | Johanna will do this |  |
| Number of missings per variable, presence of systematically missings | Anneke to create format of empty table  Johanna will do the R work |  |
| Resolve missings by passive imputation |  |  |
|  |  |  |
| ***Multiple imputation*** | | |
| Simultaneous imputation for both objective 1 and 2 |  |  |
| Impute everything, but exclude studies with systematic missings for a certain outcome |  |  |
| If covariates have many systematic missings, exclude from analysis |  |  |
| Imputation model | Check with Johanna what we used  Is timing of infection already in the imputation model (trimester of zika infection) (Johanna will check) |  |
| Timing occurrence of asymptomatic infections | Check whether this is relevant |  |
|  |  |  |
| ***Absolute and relative risk of outcomes*** | | |
| Separate for maternal and for fetal ZIKV exposure |  | Yes |
| Calculate risk difference based on absolute risks |  |  |
| Denominators differ between outcomes (Table 4) |  |  |
| Relative risks exposed vs. not exposed | To do: fix errors when we have new dataset | Yes |
| Forest plots of absolute and relative risk | To do: fix errors when we have new dataset | Yes |
| Two-stage meta-analysis of absolute and relative risk, calculate PI | To do: fix errors when we have new dataset | Yes |
| One-stage meta-analysis for relative difference: random intercept, account for confounding | To do: fix errors when we have new dataset To do: decide based on new dataset whether we will center exposure per study |  |
| One-stage meta-analysis for relative risk, random intercept | To do: fix errors when we have new dataset | Yes |
|  |  |  |
| ***Absolute risks in subgroups*** | | |
| Select subgroups (Table 3) with clinical experts |  |  |
| Stratified analysis by trimester of infection: one-stage meta-analysis |  |  |
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| ***Identify confounders and effect modifiers*** | | |
| One-stage meta-analysis of absolute risk with study-level sources of heterogeneity and random intercept and random effect for exposure -> calculate risk difference based on this.   * All confounders in 1 model * Separate model per effect modifier. Random effect on effect-modifier and exposure   Covariates need to be mean centered per study  CIs with bootstrapping | See more details in analysis plan |  |
| Two-stage meta-analysis for interaction effects   * Firth regression | See more details in analysis plan |  |
| Explore non-linearity   * Two-stage: splines, AIC * One-stage: GAMM model |  |  |
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