Diagnostic accuracy of prenatal imaging for diagnosis of congenital Zika syndrome: systematic review and meta-analysis

Appendix 2: Supplementary tables

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- Table SA4 Brain anomalies in patients with congenital zika syndrome
Search zika march 1 2022

Search "zika virus infection"[MeSH Terms] OR "zika virus infection"[Title/Abstract] OR "Congenital zika virus infection"[Title/Abstract] OR "zika infection"[Title/Abstract] OR "zika fever"[Title/Abstract] OR "zika virus disease"[Title/Abstract] AND ("ultrasound"[Title/Abstract] OR "magnetic resonance"[Title/Abstract] OR "diagnostic image"[Title/Abstract]) OR "obstetric, ultrasonography" Sort by: Most Recent

277 13:28:25#19
Search: ((("zika infection"[Title/Abstract]) OR ("zika fever"[Title/Abstract])) OR (zika virus infection[Title/Abstract])) AND ("ultrasound"[Title/Abstract]) OR ("obstetric ultrasound"[Title/Abstract]) OR ("diagnostic imaging"[Title/Abstract]) Sort by: Most Recent

2113:25:46#18
Search: (((((("zika virus infection"[MeSH Terms]) OR (zika virus infection[Title/Abstract])) OR ("Congenital zika syndrome"[Title/Abstract])) OR ("Congenital zika virus infection"[Title/Abstract] AND (y_10[Filter]))) OR ("Congenital zika virus infection"[Title/Abstract]) OR ("zika infection"[Title/Abstract])) OR ("zika fever"[Title/Abstract])) OR ("zika virus disease"[Title/Abstract]) AND (prenatal ultrasound[Title/Abstract]) OR ("magnetic resonance"[Title/Abstract]) OR ("diagnostic image"[Title/Abstract]) Sort by: Most Recent

69 13:23:38#17
Search: ((((((("zika virus infection"[MeSH Terms]) OR (zika virus infection[Title/Abstract])) OR ("Congenital zika syndrome"[Title/Abstract])) OR ("Congenital zika virus infection"[Title/Abstract] AND y_10[Filter])) OR ("Congenital zika virus infection"[Title/Abstract])) OR ("zika infection"[Title/Abstract])) OR ("zika fever"[Title/Abstract])) OR ("zika virus disease"[Title/Abstract])
Sort by: Most Recent
6,545 11:48:14#8
Search: "zika virus disease"[Title/Abstract] Sort by: Most Recent
245 11:46:18#7
Search: "zika fever"[Title/Abstract] Sort by: Most Recent
112 11:43:41#6
Search: "zika infection"[Title/Abstract] Sort by: Most Recent
289 11:42:30#4
Search: "Congenital zika virus infection"[Title/Abstract] Sort by: Most Recent
137 11:41:28#5
Search: "Congenital zika virus infection"[Title/Abstract] Filters: in the last 10 years Sort by: Most Recent
137 11:41:21#3
Search: "Congenital zika syndrome"[Title/Abstract] Sort by: Most Recent
536 11:38:43#2
Search: zika virus infection[Title/Abstract] Sort by: Most Recent
1,716 11:37:28#1
Search: "zika virus infection"[MeSH Terms] Sort by: Most Recent
6,000 11:36:39

Google

Congenital zika syndrome 506

"congenital zika infection" OR "ZikV congenital" AND "prenatal ultrasound" OR "imaging" OR "diagnostic "
INDEXTERMS("zika virus infection") OR TITLE-ABS("zika virus infection") OR (TITLE-ABS("Congenital zika virus infection") OR TITLE-ABS("zika infection") OR TITLE-ABS("zika fever") OR TITLE-ABS("zika virus disease")) AND (TITLE-ABS("ultrasound") OR TITLE-ABS("magnetic resonance") OR TITLE-ABS("diagnostic image")) OR "obstetric, ultrasonography". 125
| Author          | Sample size | Index Test | ga US(w) | US assessments | Sample size MRI | ga MRI | Operators US: definition of the index test US | Blinded operators | Equipment MRI | Interpretation MRI |
|-----------------|-------------|------------|----------|----------------|----------------|--------|-----------------------------------------------|------------------|---------------|-------------------|
| Besnard2016     | 19          | US/MRI     | NR       | Not clear      | 7              | 29.1   | Expert ob gyn, nurse                         | Not cleared       | NR            | NR                |
| Sarno2017       | 52          | US         | 24.1     | 20-24w 26-30w  | NA             | NA     | Fetal medicine                                | Blinded           | NA            | NA                |
| Rodo2019        | 72          | US/MRI     | 20.0     | monthly        | 37             | Third trimester | Detailed anatomy scan and neurosonograms | Not reported      | NR            | NR                |
| Schaub2017      | 14          | US         | 16-24    | 16-20w (12) 20-24w (10) 24-28w (5) >28w (4) | NA             | NA     | ISUOG guidelines, certified operators         | Not cleared       | NA            | NA                |
| Sohan2017       | 100         | US         | 20       | Not clear      | NA             | NA     | ISUOG guidelines, Clinician interest in fetal medicine | Blinded           | NA            | NA                |
| Mulkey2018      | 82          | US/MRI     | 25.1     | Second and third trimester | 82 | 25.1 and 30w | Radiologist after on-site protocol standardization | Blinded           | 1.5T          | Neuroradiologist and Fetal radiologist |
| SanzCortes2018  | 214         | US/MRI     | 28.4 w   | monthly        | 10             | 30.1 +/- 4.6w | Obstetricians and maternal fetal medicine specialists | Blinded           | 1.5T          | Baylor College of Medicine |
| Pomar2018       | 301         | US         | Random   | monthly        | NA             | NA     | Radiology Unit with experienced sonographers, International and national guidelines | NA               | NA            | NA                |
| Pereira2018     | 182         | US         | 26.3w    | Not clear      | NA             | NA     | Perinatologist certified by FEBRASGO. Detailed anatomy scan | Blinded           | NA            | NA                |
| Melo2018        | 11          | US/MRI     | 22.0w    | Not clear      | 6              | NR     | Fetal Medicine                               | NR               | 3T MRI or      | NR                |
| Study             | N   | Imaging Modality | Gestational Age | Timing of Postnatal MRI | Postnatal MRI Protocol | Study Design |
|-------------------|-----|------------------|-----------------|-------------------------|------------------------|--------------|
| Nogueira2018      | 216 | US/MRI           | Not clear       | 25                      | Multidisciplinary medical team | NR           |
| Pires2017         | 8   | US/MRI          | 31.3w           | Not clear               | Postnatal MRI          | Board certified operator ISUOG Guidelines | NR           |
| Carvahlo2016      | 22  | US/MRI          | 32.3w           | 19                      | Postnatal MRI          | Prenatal Guidelines ISUOG | NR           |
| Walker2018        | 56  | US               | a. 18-23 6/7w (19) <br>b. 24-27 6/7w (17) <br>c. 28-33 6/7w (38) <br>d. >34w (46) monthly | NA                      | Guidelines IG 21 standards | NR           |
| Gutierrez Sanchez2021 | 209 | US               | Not clear       | NA                      | Certified Obstetrician and Maternal Fetal Medicine specialist. ISUOG guidelines | NR           |
| Walker2020        | 95  | US               | 27.0w +/- 7.6w monthly | 7                      | Postnatal MRI if Postnatal cranial US show anomalies Intergrowth 21 standards | Blinded analysis | NR           |
| MarbanCAstro 2021 | 195 | US               | monthly         | NA                      | Experienced examiners prenatal and neursonograms | NR           |
| Coutinho2021      | 511 | US               | monthly         | NA                      | NR                     | NR           |
| Study | Setting | Population | Index text | Reference standard |
|-------|---------|------------|------------|--------------------|
| Besnard | 2016 | 2014-2015 Prospective cohort | Pregnanies during Zika outbreak with fetus with brain abnormalities | \( \text{Zika symptoms and Zika RT-PCR in amnionic fluid, cerebral anomalies} \) | 34 | 31.4 | \( \text{Prenatal brain abnormalities in US and MRI in Zika or confirmed fetus} \) | 7 | Perinatal death, Neonatal unit admission, postnatal image anomalies and clinical evidence of brainstem dysfunction |
| Rodo | 2019 | 2016-2017 Prospective cohort | Pregnant women with medical examination link | Confirmed case: serum and urine Zika RT-PCR. \* "Probable case" Zika IgM +/Zika IgG negative or indeterminate PRNT | 20 | 33.5 | \( \text{Prenatal brain abnormalities in US and MRI} \) \* Zika confirmed Serum or urine Zika RT-PCR | 2 | Newborns with clinical/radiological findings consistent with congenital Zika syndrome. Adverse perinatal outcomes: abisection or miscarriage related to Zika or CZS |
| Mulkey | 2018 | Prospective cohort | Pregnant women with symptoms | Zika laboratory confirmation | 25.1 | 38.4 | \( \text{Prenatal brain abnormalities US and MRI} \) | 3 | Newborns with clinical/radiological findings consistent of CZS |
| Sanz Cortes | 2018 | 2015-2016 Prospective | Pregnant women with symptoms or if their partners had symptoms. Women diagnosed with congenital anomalies compatible with CZS | Maternal symptoms and IgM Zika Zika RT-PCR | 21.9 | 37.8 | \( \text{Prenatal brain abnormalities in US and MRI} \) | 12 | Perinatal outcomes: neonatal anthropometry, neonatal status. Postnatal brain images (transcranial US, CT scanning and MRI) and Laboratory confirmation: RT PCR +maternal, amniotic fluid, placental or umbilical cord |
| Pomar | 2018 | 2016-2017 Prospective | Pregnant patients with Zika positive serology or positive PCR Zika | Confirmed congenital Zika infection: Zika RT-PCR at least in one sample (placenta, amniotic fluid, urine, blood or Zika virus IgM in umbilical cord/neonatal blood or cerebrospinal fluid. Maternal contamination was excluded with IgM status on day 3 and umbilical cord samples from fetal loss | \( \text{Monthly} \) | \( \text{Not clear} \) | \( \text{Prenatal brain abnormalities in US} \) | 76 | Clinical neonatologist, anthropometric measurements, neurological status. Four categories of CZS: A. Asymptomatic. B. Mild or moderate. C. Severe. D. Fetal loss and stillbirth. E. "Any adverse outcome" |
| Guiterrez Sanchez | 2021 | 2015-2017 Prospective | Pregnant patients with clinical symptoms with Zika PCR | Confirmed congenital Zika infection: Zika RT-PCR in blood, urine or amniocentesis sample. Negative case: Zika RT-PCR negative | 31.5 | 38 | \( \text{Prenatal brain abnormalities in US} \) | 32 | Intraterine Growth restriction, Birth outcome, aqger score |
| Walker | 2020 | 2016-2017 Retrospective | Pregnant women screening Questionnaire. Tertiary care center in Miami | Confirmed maternal infection: Zika RT-PCR in blood, Zika IgM + or equivocal, Zika PRNT >10 titer | 29.8 | 27.4 | \( \text{US Confirmed case+Positive Zika RT-PCR in neonatal serum, urine or cerebrospinal fluid} \) | 24 | Clinical neonatal evaluation, postnatal US, MRI if indicated and additional auditory screening and visual testing. Fetal body ratio (HC:FL) at 10 centile and at 3rd centile |
| Walker | 2018 | 2016-2017 Retrospective | Pregnant women from an area with local Zika transmission at Columbia Medical Center screening | Confirmed maternal infection: Zika RT-PCR in blood, Zika IgM + or equivocal, Zika PRNT >10 titer | \( \text{NR} \) | 57.4 | \( \text{US Confirmed case+Positive Zika RT-PCR in neonatal serum, urine or cerebrospinal fluid} \) | 3 | Clinical neonatal evaluation, postnatal US, MRI if indicated and additional auditory screening and visual testing. Brain anomalies, Intrauterine growth restriction. Fetal body ratio (HC:FL:AC:FL) <10 percentile |
| Pereira | 2018 | 2015-2016 Prospective | Pregnant women with Zika RT-PCR Blood or urine that underwent 1 prenatal US | Confirmed case: serum and urine Zika RT-PCR | 26.3 | 38.6 | \( \text{Abnormal ultrasound; at least one of these parameters: any structural anomaly, abnormal fetal growth measurements, fetal growth restriction or macrosomia, abnormal umbilical artery, abnormal MCA doppler measurements, abnormal amnionic fluid assessment or placentalomegaly. Categorization Zika related anomaly and another with unknown significance with Zika} \) | 37 | Composite perinatal outcome defined by perinatal death, an abnormal finding of neonatal examination or abnormal finding on postnatal neuroimaging examinations. Perinatal outcomes: NICU admission. Clinical examinations were performed by neonatologist, pediatric infectious disease specialist, geneticists, and neurologists |
| Study                | Setting     | year | study period | study temporality | inclusion criteria | Pregnant women with Zika symptoms and with symptoms | Pregnant women with confirmed or presumed Zika infection | Pregnant women with Zika infection | Suspected Maternal Zka definition | GA at inclusion (weeks) | GA at birth (weeks) | Definition of CZS | Number of CZS fetuses | Included outcomes                                                                 |
|---------------------|-------------|------|--------------|-------------------|--------------------|-----------------------------------------------------|-----------------------------------------------------|-------------------------------|--------------------------|----------------------|---------------------|---------------------|-----------------------|-----------------------------------------------------------------------------------|
| Nogueira            | Prospective | 2018 | 2015-2016    | Retrospective     | Pregnant with acute Zika symptoms | 216 | ZIKV RT-PCR positive | Maternal symptoms And IgM Zika or Zika RT-PCR as confirmed by ZIKA virus neutralization | Retrospective | NR | 37.5 | US Preterm brain abnormalities ZIKV RT-PCR positive umbilical cord blood and urine | 18 | Newborns with clinical/radiological findings consistent of CZS Adverse outcomes: brain anomalies, premature birth and abnormal otoacoustic emission |
| Pires               | Prospective | 2017 | 2015-2016    | Retrospective     | Pregnant with confirmed or presumed Zika infection | 8 | Maternal symptoms And IgM Zika or Zika RT-PCR as confirmed by ZIKA virus neutralization | Maternal symptoms And IgM Zika or Zika RT-PCR as confirmed by ZIKA virus neutralization | Retrospective | 31.3 | 38.0 | US Preterm brain abnormalities confirmed ZIKV RT-PCR | 8 | Birth outcomes: neonatal Postnatal brain images (transcranial US, CT scanning and MRI) and Laboratory confirmation: RT PCR + amniotic fluid, placental or umbilical cord |
| Schaub              | Retrospective | 2017 | 2016         | Retrospective     | Pregnant women with Zika infection in their first or second trimester and with fetal brain anomalies | 42 | Confirmed ZIKV RT-PCR in maternal blood or positive Zika Virus IgM (ratio >3) or IgG seroconversion confirmed by Zika virus neutralization | Confirmed: Zika virus RNA RT-PCR at fetal/neonatal sample, placenta, amniotic fluid, cerebrospinal fluid, urine and blood | Retrospective | 16 | 27.4 | Confirmed congenital Zika infection: Zika virus RNA RT-PCR at fetal/neonatal sample, placenta, amniotic fluid, cerebrospinal fluid, urine and blood | 14 | Brain anomalies and autopsy |
| Sohan               | Retrospective | 2017 | 2016         | Retrospective     | Pregnant women with serological evidence of Zika infection (RT-PCR + and IgM + (2) or with symptoms | 100 | Confirmed maternal infection: +Zika RT-PCR in blood; ZIKV IgM + or equivocal; ZIKV PRNT >10 titer Negative ZIKV IgM, positive or equivocal dengue IgM and ZIKV PRNT > 10 titer | No laboratory zika confirmation on fetal/neonatal sample were performed | Retrospective | 20 | 27.6 |  | 6 | Birth outcomes, Brain anomalies |
| Meilo               | Prospective | 2016 | 2015-2016    | Retrospective     | Pregnant women with Zika symptoms in the first half of pregnancy and fetus with some brain anomalies | 11 | Confirmed or presumed zika infection according to Brazilian Ministry of Health guidelines | Confirmed cases: laboratory evidence of zika virus infection (IgM or ZIKV RT-PCR +) | Retrospective | 22 | 39.0 | Suspected cases: Brain anomalies, maternal history of Zika symptoms and strong IgG anti ZIKA virus | 11 | Amniotic fluid, Cord Blood, placenta, neonatal tissues collected postmortem |
| Carvalho            | Retrospective | 2016 | 2015-2016    | Retrospective     | Pregnant women with Zika infection and with probable fetuses with microcephaly | 22 | Confirmed maternal infection: +Zika RT-PCR in blood; ZIKV IgG IgM Zika antibodies | Confirmed: ZIKV RT-PCR + in amniotic fluid or neonatal sample | Retrospective | 32.3 | NR | Confirmed: ZIKV RT-PCR + in amniotic fluid or neonatal sample | 19 | Birth outcome, Brain postnatal abnormalities assessment with CT scans and MRI Perinatal death |
| Marban Castro       | Prospective | 2021 | 2016-2020    | Retrospective     | Pregnant women that travelled to endemic areas or partner | 195 | Confirmed maternal infection: +Zika RT-PCR in blood; ZIKV IgG IgM Zika antibodies | Confirmed case: Positive ZIKV RT PCR in neonatal serum, urine or cerebrospinal fluid Probable case: IgM Zika positive or equivocal with negative ZIKV RT PCR | Retrospective | NR | 38.5 | Confirmed case: Positive ZIKV RT PCR in neonatal serum, urine or cerebrospinal fluid Probable case: IgM Zika positive or equivocal with negative ZIKV RT PCR | 4 | Clinical neonatal evaluation, postnatal US, MRI if indicated and additional auditory screening and visual testing. Brain anomalies |
| Sarno               | Prospective | 2017 | 2015-2016    | Retrospective     | Pregnant women with Zika symptoms | 60 | Molecular and epidemiological link with ZIKV: ZIKV RT-PCR +, sexual contact with confirmed case, history of symptoms; residing or travelling to an area with ongoing transmission | Confirmed case: ZIKV RT-PCR + amniocentesis or fetal brain tissue | Retrospective | 24.1 | NR | Confirmed case: ZIKV RT-PCR + amniocentesis or fetal brain tissue | 52 | Clinical neonatal evaluation and postnatal medically indicated cranial/ultrasound/Eye examination |
| Coutinho            | Prospective | 2018 | 2015-2016    | Retrospective     | Pregnant women with ZIKV RT-PCR Blood/urine | 511 | Pregnant women with ZIKV RT-PCR Blood/urine | Anti IgM antibodies +; ZIKA RNA + | Retrospective | NR | 39 |  | Abnormal perinatal outcome: abortion or miscarriage related to ZIKV or CZS Stillbirth Severe Brain anomalies Neonatal deaths | 19 | Abnormal perinatal outcome: abortion or miscarriage related to ZIKV or CZS Stillbirth Severe Brain anomalies Neonatal deaths |
Tables 2 x 2 – Studies included in meta-analysis

Besnard 2016

Population assessed in the study was comprised by 19 cases of congenital cerebral malformations. CZS was not examined for every case “For some cases with of non-microcephalic, congenital abnormalities we were not able to prove or exclude ZIKV infection retrospectively”. Data extracted took into account malformation and report of ZIKV infection, based on PCR evaluation or mother reporting symptoms during pregnancy.

|                          | N | Source                                                                 |
|--------------------------|---|----------------------------------------------------------------------|
| TP (abnormal prenatal imaging + zika confirmation) | 7 | According to figure 4, cases with abnormal prenatal US and confirmation of ZIKV infection (4) and abnormal prenatal US+ mother experiencing symptoms of ZIKV infection + medical termination of pregnancy (3). |
| FP (abnormal prenatal imaging + zika negative)      | 6 | According to figure 4, cases with abnormal prenatal US and ZIKV RT-PCR negative (3) and abnormal prenatal US+ no symptom (3; cases 7, 9 and 12). |
| FN (normal prenatal imaging + abnormal postnatal outcome) | 0 | There is no report of normal prenatal US and brain abnormalities related to ZIKV infection. |
| TN (normal prenatal and mothers reporting no ZIKV infection symptoms/no ZIKV neutralising antibodies) | 6 | According to figure 4, prenatal US did not show brain abnormalities in 6 cases, 5 cases reported under group 3 and 1 under group 1b. It is mentioned that cases on group 3 had no ZIKV neutralising antibodies and no symptoms during pregnancy and laboratory tests were normal for the 1 case on group 1b, and the mother was asymptomatic. |
The study reported 19 cases of microcephaly, ALL detected by prenatal US. Thus, FN and TN = zero.

According to the study:

- “a direct association between fetal malformations and Zika virus could not be established as placenta investigations were not performed”,
- “In our series other congenital infections such as TORCH and syphilis were ruled out in all pregnant women, and genetic syndromes were excluded by clinical examination by a medical geneticist at birth.”
- “A limitation of our series is that only cases with CNS malformations detected on prenatal ultrasound were included. Thus, the congenital anomalies detected in our series may possibly represent the end-point of the spectrum of intrauterine Zika infection.”

Thus, all cases were classified as TP.
Study included 82 pregnant women with Zika virus infection, 3 cases of severe fetal brain abnormalities were prenatal imaging (TP). From the 79 cases with normal prenatal imaging, postnatal imaging was performed only in 61 cases to confirm CSZ. Abnormal postnatal imaging was observed in 23 newborn (FN). TN: 61 - 23 = 38

| Source                                                                 | N  |
|-----------------------------------------------------------------------|----|
| TP (abnormal prenatal imaging + postnatal confirmation)               | 3  |
| FP (abnormal prenatal imaging + no postnatal confirmation)            | 0  |
| FN (normal prenatal imaging + abnormal postnatal imaging)             | 23 |
| TN (normal prenatal and postnatal imaging)                            | 38 |
Data was extracted from Supplementary Table 5 (abnormal prenatal US) and Supplementary Table 6 (confirmation of CSZ - Abnormal neurological evaluation). Abnormal prenatal US was observed in the whole population (ZIKV-positive and ZIKV-negative).

|          | N   | Source                                                                 |
|----------|-----|------------------------------------------------------------------------|
| TP (abnormal prenatal US + ZIKV RNA detected) | 18  | Data was extracted from Supplementary Table 5 (abnormal prenatal US) and Supplementary Table 6 (confirmation of CSZ - Abnormal neurological evaluation). Abnormal prenatal US was observed in the whole population (ZIKV-positive and ZIKV-negative). |
| FP (abnormal prenatal US + No ZIKV RNA detected) | 0   |                                                                         |
| FN (normal prenatal US + ZIKV RNA detected)    | 0   |                                                                         |
| TN (normal prenatal US + No ZIKV RNA detected)  | 36  |                                                                         |
|                   | N  | Source                                      |
|-------------------|----|---------------------------------------------|
| TP (abnormal       | 22 | Data extracted from:                        |
| ultrasonography +  |    | Figure. Flow of Patients Through the Study  |
| abnormal          |    |                                             |
| neonatal outcome) |    |                                             |
| FP (abnormal       | 15 |                                             |
| ultrasonography +  |    |                                             |
| normal             |    |                                             |
| neonatal outcome)  |    |                                             |
| FN (normal         | 23 |                                             |
| ultrasonography +  |    |                                             |
| abnormal           |    |                                             |
| neonatal outcome)  |    |                                             |
| TN (normal         | 32 |                                             |
| ultrasonography +  |    |                                             |
| normal             |    |                                             |
| neonatal outcome)  |    |                                             |
|        | N   | Source                                                                                                                                 |
|--------|-----|----------------------------------------------------------------------------------------------------------------------------------------|
| TP (abnormal prenatal US + Confirmed congenital Zika virus infection) | 27  | cZIKV positive babies that had abnormal prenatal US listed on appendix 4 (15 cZIKV positive babies had normal prenatal US that were subtracted from the 42 cZIKV positive babies – figure 2) |
| FP (abnormal prenatal US + Negative fetal/neonatal Zika virus testing) | 22  | cZIKV negative babies with abnormal prenatal US (appendix 4)                                                                                                                                  |
| FN (normal prenatal US + Confirmed congenital Zika virus infection)    | 49  | 15 cZIKV positive babies that had normal prenatal US (appendix 4) + 33 cZIKV positive babies that were asymptomatic (figure 2)                                                               |
| TN (normal prenatal US + Negative fetal/neonatal Zika virus testing)    | 193 | 5 normal prenatal US (appendix 4) + 188 Negative fetal/neonatal Zika virus testing that were asymptomatic                                                                                       |
|                  | N  | Source                                                                 |
|------------------|----|------------------------------------------------------------------------|
| TP (abnormal prenatal US + CZS positive cases) | 12 | All 12 fetuses showed significant brain findings during MRI and ultrasound evaluation. |
| FP (abnormal prenatal US + negative CZS)     | 0  | CSZ was not confirmed for 202 subjects that were initially referred. The study did not mention any information on US findings for this group. |
| FN (normal prenatal US + CZS positive cases) | 0  | All 12 fetuses showed significant brain findings during MRI and ultrasound evaluation, no observation of normal US in CZS positive cases. |
| TN (CZS negative with normal US)             | 202| CSZ was not confirmed for 202 subjects that were initially referred. The study did not mention any information on US findings for this group. |
The study reports abnormal ultrasound findings of 52 fetuses with Zika virus-related microcephaly. Follow-up data were available in 35 of the 52 fetuses classified as having ZIKV-related microcephaly according to WHO criteria (TP).

| Category | N   | Source |
|----------|-----|--------|
| TP (abnormal prenatal imaging + fetus classified as having ZIKV-related microcephaly after birth) | 35  |        |
| FP (abnormal prenatal imaging + fetus not classified as having ZIKV-related microcephaly after birth) | 17  | The study reports abnormal ultrasound findings of 52 fetuses with Zika virus-related microcephaly. Follow-up data were available in 35 of the 52 fetuses classified as having ZIKV-related microcephaly according to WHO criteria (TP). |
| FN (normal prenatal imaging + fetus classified as having ZIKV-related microcephaly after birth) | 0   |        |
| TN (normal prenatal imaging + fetus not classified as having ZIKV-related microcephaly after birth) | 0   |        |
| N | Source |
|---|---|
| **TP (abnormal prenatal US + CZS positive cases)** | 2 | In the “confirmed infection” group there were two cases of congenital disease (page 633.e6) and “MRI confirmed the abnormal ultrasound findings in the two CZS cases (p 0.042) (please see supplementary material).” |
| **FP (abnormal prenatal US + no CZS)** | 1 | Second-trimester ultrasound was normal in all except three cases: two cases of brain malformations in the confirmed group (2/8, 25%), both consistent with CZS (case 1 and 8, Table 2), and one case of congenital heart defect in the probable infection group (p = 0.004). |
| **FN (normal prenatal US + CZS positive cases)** | 0 | Only 2 CZS cases that were confirmed by US. |
| **TN (CZS negative with normal US)** | 68 | CZS was not confirmed in 7 out of 9 on “confirmed infection” and in 62 on the “Probable infection” group (1 case had abnormal US) |
The study reported 14 cases of pregnant women with confirmed Zika virus infection AND fetal abnormalities of the brain. Abnormal ultrasound scans was reported for ALL cases. Positive Zika virus RT-PCR is reported for ALL fetuses.

| Source | N  |
|--------|----|
| TP (abnormal prenatal imaging + zika confirmation) | 14 |
| FP (abnormal prenatal imaging + zika negative)   | 0  |
| FN (normal prenatal imaging + zika confirmation) | 0  |
| TN (normal prenatal and no zika confirmation)   | 0  |
Definitions of fetal brain anomalies

Definitions of the anomalies in fetal brain reported: Three studies classify fetal brain findings in major and minor anomalies (37, 43, 46). Major anomalies associated to CZS included: major CNS abnormality, like agenesia of corpus callosum, brainstem dysgenesis and migration disorders. Minor findings included: abnormal doppler, umbilical artery and middle cerebral artery; fetal growth restriction (FGR), amniotic fluid anomalies, oligohydramnios, and polyhydramnios; choroid plexus cyst. One of the studies did not report any brain abnormalities definitions at all (35).

Fetal microcephaly: Mild to moderate microcephaly was determined based on the head circumference, but the criterion varied across studies. Of the 18 included studies, 6 (33.3%) used a head circumference smaller than three standard deviations below the mean to define microcephaly. One study defined severe microcephaly as a head circumference smaller than three standard deviations below the mean of the gestational age or smaller than two standard deviations below the mean, along with some other CNS findings. Five studies used the CDC definition of microcephaly as a head circumference smaller than the 3rd percentile for gestational age (33). Different HC growth curves were used to classify neonatal microcephaly: INTERGROWTH-21st (IG-21) (4, 47, 49), Fenton curves (36), IG-21 and the World Health Organization (WHO) Fetal Growth chart (WHO-FGC) (29,48). Sanz Cortes, et al compare neonatal head circumferences with both IG-21 and Fenton curves (4).

Fetal ventriculomegaly: Fetal ventriculomegaly definition varied across the studies. Majority of the studies classified ventriculomegaly as severe, above 15mm (4, 36-40). Other studies classified as a mild ventriculomegaly when the level of the atria measures between 10-12mm; Sarno et al (33), classified a mild anomaly when atria <15mm. One study used to cut off >10mm to describe ventriculomegaly (42).

Growth abnormalities: Pires et al estimated fetal weight (EFW) with the Hadlock 3 formula (39); Pereira, Mulkey defined FGR as fetal weight <10 percentile with Hadlock formula (35,42) ; IG-21 was used by (4,36). One study reported femur sparring pattern of fetal growth restriction using fetal body ratios and IG-21 and did not use EFW to make the definition (47). Pereira small for gestational age neonate (SGA) was defined by IG-21 standards-Doppler studies. The pulsatility index of the umbilical artery and middle cerebral artery (MCA) were defined as abnormal if 1) greater than the 95th percentile or 2) if the peak systolic velocity of the MCA was greater than 1.5 multiples of the median.
### Table SA4. Brain anomalies in confirmed congenital zika syndrome

| Study                | Confirmed CZS | Ventriculomegaly severe (%) | Dysgenesia callosal (%) | Periventricular Calcifications (%) | Migration disorders (%) | Microcephaly (%) | Cortical anomalies (%) | Decreased brain volume (%) | Heterotopia Nodular | Others                        |
|----------------------|---------------|-----------------------------|-------------------------|-----------------------------------|-------------------------|------------------|------------------------|-------------------------------|----------------------|-----------------------------|
| Besnard              | 19            | 7 (37%)                     | 7 (37%)                 | 8 (42%)                           | 6 (31%)                 | 6 (31%)          |                        |                               |                      |                             |
| Mulkey               | 3             | 1                           |                         |                                   |                         |                  |                        |                               |                      |                             |
| Sanz Cortes          | 12            | 12 (100%)                   | 11 (92%)                | 7/9 (78%)                         | 8/9 (89%)               |                  |                        |                               |                      |                             |
| Pomar                | 76            |                             |                         | 5                                 |                         |                  |                        |                               |                      |                             |
| Gutierrez Sánchez    | 32            |                             |                         |                                   |                         |                  |                        |                               |                      |                             |
| Walker 2018          | 3             |                             |                         |                                   |                         |                  |                        |                               |                      |                             |
| Walker 2020          | 24            |                             |                         |                                   |                         |                  |                        |                               |                      |                             |
| Pereira              | 37            | 6 (16%)                     | 2 (5%)                  | 9 (24%)                           | 7 (19%)                 |                  |                        |                               |                      |                             |
| Nogueira             | 18            | NR                          | NR                      | NR                                | NR                      | NR               | NA                     | NA                            |                      |                             |
| Pires                | 8             |                             |                         |                                    |                         |                  |                        |                               |                      | 8 (100%)                     |
| Schaub               | 14            | 8                           | 10 (71%)                | 10 (71%)                          |                         |                  |                        |                               |                      |                             |
| Sohan                | 9             | 2                           |                         |                                    |                         |                  |                        |                               |                      |                             |
| Melo                 | 11            | 10 (90%)                    | 1                       |                                    |                         |                  |                        |                               |                      | 9 (82%)                     |
| Carvahlo             | 19            | 10 (53%)                    | 15 (79%)                |                                    |                         |                  |                        |                               |                      |                             |
| Marban Castro        | 4             |                             |                         |                                    |                         |                  |                        |                               |                      | 1 (subependymal pseudocyst) |
| Sarno                | 52            | 48 (65%)                    | 2                       | 23 (44%)                          | NR                      | 46 (88%)         | NR                     | NA                            |                      | NA                          |
| Coutinho             | 20            |                             |                         |                                    |                         |                  |                        |                               |                      |                             |
| Rodo                 | 2             |                             |                         |                                    |                         |                  |                        |                               |                      |                             |