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Zika virus detection in amniotic fluid and Zika-associated birth defects

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Abstract

BACKGROUND—Zika virus infection during pregnancy can cause serious birth defects, which include brain and eye abnormalities. The clinical importance of detection of Zika virus RNA in amniotic fluid is unknown.

OBJECTIVE—The purpose of this study was to describe patterns of Zika virus RNA testing of amniotic fluid relative to other clinical specimens and to examine the association between Zika virus detection in amniotic fluid and Zika-associated birth defects. Our null hypothesis was that Zika virus detection in amniotic fluid was not associated with Zika-associated birth defects.

STUDY DESIGN—We conducted a retrospective cohort analysis of women with amniotic fluid specimens submitted to Colombia's National Institute of Health as part of national Zika virus surveillance from January 2016 to January 2017. Specimens (maternal serum, amniotic fluid, cord

blood, umbilical cord tissue, and placental tissue) were tested for the presence of Zika virus RNA with the use of a singleplex or multiplex real-time reverse transcriptase-polymerase chain reaction assay. Birth defect information was abstracted from maternal prenatal and infant birth records and reviewed by expert clinicians. Chi-square and Fisher's exact tests were used to compare the frequency of Zika-associated birth defects (defined as brain abnormalities [with or without microcephaly, but excluding neural tube defects and their associated findings] or eye abnormalities) by frequency of detection of Zika virus RNA in amniotic fluid.

RESULTS—Our analysis included 128 women with amniotic fluid specimens. Seventy-five women (58%) had prenatally collected amniotic fluid; 42 women (33%) had amniotic fluid collected at delivery, and 11 women (9%) had missing collection dates. Ninety-one women had both amniotic fluid and other clinical specimens submitted for testing, which allowed for comparison across specimen types. Of those 91 women, 68 had evidence of Zika virus infection based on detection of Zika virus RNA ≥in 1 specimen. Testing of amniotic fluid that was collected prenatally or at delivery identified 39 of these Zika virus infections (57%; 15 [22%] infections were identified only in amniotic fluid), and 29 infections (43%) were identified in other specimen types and not amniotic fluid. Among women who were included in the analysis, 89 had pregnancy outcome information available, which allowed for the assessment of the presence of Zika-associated birth defects. Zika-associated birth defects were significantly (P<.05) more common among pregnancies with Zika virus RNA detected in amniotic fluid specimens collected prenatally (19/32 specimens; 59%) than for those with no laboratory evidence of Zika virus infection in any specimen (6/23 specimens; 26%), but the proportion was similar in pregnancies with only Zika virus RNA detected in specimens other than amniotic fluid (10/23 specimens; 43%). Although Zika- associated birth defects were more common among women with any Zika virus RNA detected in amniotic fluid specimens (ie, collected prenatally or at delivery; 21/43 specimens; 49%) than those with no laboratory evidence of Zika virus infection (6/23 specimens; 26%), this comparison did not reach statistical significance (P=07).

CONCLUSION—Testing of amniotic fluid provided additional evidence for maternal diagnosis of Zika virus infection. Zika-associated birth defects were more common among women with Zika virus RNA that was detected in prenatal amniotic fluid specimens than women with no laboratory evidence of Zika virus infection, but similar to women with Zika virus RNA detected in other, nonamniotic fluid specimen types.

Keywords

amniotic fluid; birth defect; microcephaly; PCR; pregnancy; Zika virus; ZIKV disease

Zika virus (ZIKV) is a flavivirus that is transmitted primarily through the bite of an infected *Aedes* species mosquito, but sexual and vertical transmission (from mother to fetus) are well- documented.[1–3] Although symptomatic ZIKV infection is typically mild (rash, fever, headache, arthralgia, myalgia, and nonpurulent conjunctivitis) and selflimited, an estimated 80% of ZIKV infections are asymptomatic.[1,4] ZIKV is a teratogen; infection during pregnancy is associated with a 5—10% risk of fetal brain and eye abnormalities and sequelae of central nervous system dysfunction, such as arthrogryposis.[5—7]

A ZIKV outbreak began in the Americas in early 2015. Since August 2015, the Colombian National Institute of Health (Instituto Nacional de Salud [INS]) has maintained ongoing passive, national surveillance for symptomatic ZIKV disease. In October 2015, INS implemented mandatory reporting of symptomatic ZIKV disease. Beginning on December 24,2015, symptomatic ZIKV disease was defined as fever and rash and ≥ 1 of the following symptoms: nonpurulent conjunctivitis, arthralgias, myalgia, headache, pruritus, or malaise.[8] During the 2015—2016 ZIKV outbreak, Colombia reported >106,000 symptomatic cases of suspected ZIKV infection, which included >18,000 cases in pregnant women.[9] Colombia had one of the highest ZIKV disease burdens in the world during the outbreak, second only to Brazil. INS has also conducted mandatory passive national surveillance for major birth defects since 2010.[10]

Based on a preliminary report, the prevalence of microcephaly increased 4fold during the Colombian ZIKV epidemic, from 2.1 per 10,000 births in 2015 to 9.6 in 2016.[11]

Acute ZIKV infection is diagnosed by detection of ZIKV RNA in whole blood, serum, or urine, ideally collected within 1 week of symptom onset, although ZIKA RNA has been estimated to persist for up to 40 days in pregnant women.[12—16] ZIKV antibodies in serum can also indicate recent infection; it can be difficult to use antibody testing to distinguish between acute and previous ZIKV infection and from other common flaviviruses, such as dengue.[14,17—19] Current Centers for Disease Control and Prevention (CDC) guidelines for diagnosis of ZIKV and dengue infection in symptomatic pregnant women are to collect serum and urine specimens within 12 weeks of symptom onset, ideally as close to symptom onset as possible, for identification of ZIKV and/or dengue RNA or immunoglobulin M antibodies.[14] ZIKV RNA has been detected in amniotic fluid, placenta, umbilical cord tissue and blood, tissues from fetal losses, and infant cerebrospinal fluid (CSF).[20—23] Early recommendations for the management of ZIKV during pregnancy were derived from experience with prenatal cytomegalovirus infection.[24,25] Although amniocentesis is useful for diagnosing fetal cytomegalovirus infection, the clinical importance of testing amniotic fluid to detect ZIKV RNA remains unclear. Most previous studies that have documented ZIKV detection in amniotic fluid have been small (≤20 women) case series,[23,26–30] with few of them describing ZIKV test results of amniotic fluid relative to other specimens.[23,26] Studies have also largely drawn from pregnant women with laboratory-confirmed ZIKV infection whose fetuses had prenatally detected Zika-associated birth defect(s).[22,26,31]

Our objectives were to assess the clinical importance of ZIKV testing of amniotic fluid by (1) describing patterns of ZIKV test results from amniotic fluid relative to other specimens and (2) examining the association between ZIKV detection in amniotic fluid and the identification of Zika-associated birth defects.

Materials and Methods

Design and rationale

We conducted a retrospective cohort analysis of women with amniotic fluid specimens submitted to INS for ZIKV testing from January 2016 to January 2017. All specimens were

collected from pregnant women, based on the clinical judgment of their healthcare providers.

We abstracted maternal prenatal and infant birth hospitalization records for women from the 4 Colombian departments with the most amniotic fluid specimens submitted for testing. In addition, we obtained ZIKV test results for other clinical specimens that were submitted to INS as part of routine surveillance from the national surveillance system. Our null hypothesis was that ZIKV detection in amniotic fluid was not associated with the presence of Zika-associated birth defects.

Laboratory methods

INS's National Virology Reference Laboratory conducted ZIKV testing of amniotic fluid and other specimens according to established protocols.[32,33] Samples were frozen (—20° C) on arrival at Departmental laboratories and were shipped within 24 hours of collection to INS, maintaining cold-chain. At INS samples were stored at —80° C until further processing. A completed notification form about ZIKV symptom onset was transmitted to the national public health surveillance system.[34] Molecular detection of ZIKV RNA was performed by 2 methods: (1) an in-house single target real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) assay for ZIKV, with the use of previously published primers and probes and validated at INS (singleplex assay),[33] and (2) the Trioplex rRT-PCR assay (Trioplex; CDC, Atlanta, GA), for ZIKV, Chikungunya, and Dengue viruses.[32] All amniotic fluid specimens were tested with the use of the singleplex assay with 140 μL input volumes for extraction. Serum, urine, and tissue specimens were tested with singleplex or Trioplex, depending on assay availability at the time of specimen collection. In March 2016, the Trioplex kit received Emergency Use Authorization from the Food and Drug Administration. An rRT-PCR cycle threshold (C_T) value <38 was considered "positive," and values ≥38 were considered to be "negative" for ZIKV, as stated in the Emergency Use Authorization. For quality assurance, repeat ZIKV testing with Trioplex was conducted on a subset of 50 amniotic fluid specimens at CDC.

Maternal and neonatal information

Specimen type (maternal amniotic fluid, serum, urine, cord blood, cord tissue, placenta, and infant CSF), collection date, ZIKVRNAdetection based on rRT-PCR result (ie, ZIKVþ or ZIKVe), and CT value (in RT-PCR, CT values are inversely proportional to viral load) were obtained from INS's laboratory. Maternal symptomatic ZIKV disease, date of ZIKV symptom onset, maternal age at time of notification, and pregnancy outcome (ie, pregnancy loss [miscarriage at <20 weeks gestation or fetal death at ≥20 weeks gestation] or livebirth) were obtained from the surveillance system, when available. Data were obtained from maternal and fetal/ infant medical records on pregnancy gestational dating (ie, last menstrual period date, estimated date of delivery, and gestational age at birth), date of birth/end of pregnancy, prenatal ultrasound dates and findings (from the first dating ultrasound scan, first ultrasound scan with abnormal findings, and final ultrasound scan, when available), fetal magnetic resonance imaging date and findings, transfontanellar ultrasound date and findings, and infant physical examination findings at delivery.

Zika-associated birth defects

Zika-associated birth defects were identified based on information abstracted from prenatal ultrasound scans, newborn assessments, and pre- or postnatal imaging. They were defined as brain abnormalities (with or without microcephaly; excluding neural tube defects and their associated findings) or eye abnormalities, identified prenatally (for pregnancy losses) or confirmed postnatally (for live births).[7,35] At least 2 subject matter experts (R.R.G. and M.D.) reviewed each medical record using a standardized tool and well- established criteria to categorize Zika- associated birth defects.[7,35]

Data analysis

We described characteristics of pregnant women (age, gestational age at symptom onset, and pregnancy outcome) and specimens tested for ZIKV (gestational timing of specimen collection, ZIKV rRT-PCR results, and C_T values). We compared the ZIKV results of amniotic fluid with other specimens, accounting for gestational timing of specimen collection. We described the proportion of pregnancies with evidence of Zika- associated birth defects by presence and timing of ZIKV+ results in any clinical specimen, and in amniotic fluid specifically, and by ZIKV symptom onset. Because amniotic fluid specimens that were obtained on delivery date might reflect misclassification of specimen collection dates or cross-contamination with maternal blood, we conducted subanalyses among pregnancies with prenatally collected amniotic fluid, when feasible. Chi-square and Fisher's exact tests, for small sample sizes, were used to compare frequencies, and Kruskal- Wallis tests were used to compare median values. Analyses were conducted with the use of SAS software (version 9.4; SAS Institute Inc, Cary, NC). This analysis was submitted to the CDC's Human Subject officials and was deemed public health practice and was exempt from Institutional Review Board review.

Results

Characteristics of the analytic sample

From January 2016 to January 2017, INS's virology laboratory received 136 amniotic fluid specimens from 128 women (8 women had 2 specimens) for ZIKV testing from the 4 departments that were included in this analysis. Most women (55%) were aged 20—29 years (Table 1). ZIKV symptom onset was comparable across the 3 trimesters of pregnancy (23—24%) but was unknown for 21% of women. Most pregnancies (68%) resulted in a live birth (including 6 neonatal deaths); 18% resulted in a pregnancy loss, and 14% had unknown pregnancy outcomes.

Amniotic fluid specimens

Over one-half of initial amniotic fluid specimens (75/128; 59%) were collected during the second or third trimester of pregnancy; one-third of the specimens were collected on the day of delivery (Table 1). Maternal serum, umbilical cord tissue and blood, and placenta were also commonly tested for ZIKV, but only 4 maternal urine and 8 infant CSF specimens were tested. Most women (89/128; 70%) had ≥1 ZIKV+specimen, which included 47% with ZIKV+ amniotic fluid specimens (Table 1). Among ZIKV+ specimens, amniotic fluid C_T

values were significantly lower, which means a higher viral load, compared with umbilical cord blood C_T values and borderline significantly lower (P=.051) compared with serum C_T values (Supplemental Figure 1, A). ZIKV+ amniotic fluid specimen C_T values were significantly lower (which indicates a higher viral load) for women with first, compared with third, trimester symptom onset (P<.05; Supplemental Figure 1, B). Differences in C_T values by symptom onset were not explained by amniotic fluid specimen collection timing (data not shown).

ZIKV symptom onset date was available for 44 women with prenatally collected amniotic fluid and ≥1 ZIKV+ specimen. Symptom onset to amniotic fluid specimen collection for ZIKV+ amniotic fluid specimens (n=31) was shorter (median: 67 days; range: 0—184 days), although not statistically different, from ZIKV— specimens (n=13; median, 122; range, 0—163; P>.05; Supplemental Figure 2).

ZIKV detection in amniotic fluid compared with other specimen types

Eighty-nine women had ≥1 ZIKV+ specimen, of which 68 women had both an amniotic fluid and ≥1 other specimen type collected. Amniotic fluid testing identified 39 of 68 ZIKV infections (57%; 15/68 infections [22%] were identified only in amniotic fluid). ZIKV was not detected in amniotic fluid in 29 of 68 women (43%); however, ZIKV was detected in other specimen types for these patients (Figure). Sixteen patients had serum and amniotic fluid specimens collected on the same day, which included 12 that were ZIKV+ in at least 1 specimen (Table 2). Of those, most (9/12; 75%) were ZIKV+ in amniotic fluid but not in serum, and 25% of patients (3/12) were ZIKV+ in both serum and amniotic fluid. None were ZIKV+ in serum but not in amniotic fluid. Among 19 women with amniotic fluid specimens (collected prenatally or at delivery) after a ZIKV+ serum sample, 8 of 19 women (42%) were subsequently ZIKV+ in amniotic fluid; 11 of 19 women (58%) were ZIKV— in amniotic fluid. (When restricted to 15 women with prenatally collected amniotic fluid, 7/15 amniotic fluid samples [47%] were ZIKV+ and 8/15 [53%] were ZIKV—.) Among women with ≥1 ZIKV+ amniotic fluid or other specimen collected on the day of delivery, amniotic fluid was ZIKV+ and the other specimen type ZIKV— for 2 of 11 (18%), 1 of 8 (13%), and 0 of 9 (0%) women with umbilical cord tissue, umbilical cord blood, or placenta tested, respectively (Table 2). No concordance between amniotic fluid and infant CSF was observed among 4 mother-infant pairs with at least 1 ZIKV+ amniotic fluid or CSF specimen (2 pairs had ZIKV+ amniotic fluid and ZIKV— infant CSF; 2 had ZIKV— amniotic fluid and ZIKV + infant CSF).

Zika-associated birth defects

Eighty-nine women had pregnancy outcome information available to examine Zika-associated birth defects (Supplemental Figure 3). Of these, 37 of 89 women (42%) had an infant/fetus with a Zika-associated birth defect, which was significantly more common among pregnancy losses (15/23; 65%) than live births (22/66; 33%; P<.05; Table 3). Compared with pregnancies among women without any ZIKV+ specimens (6/23; 26%), Zika-associated birth defects were more common among pregnancies to women with (1) ≥1 ZIKV+ specimen (31/66; 47%), (2) ZIKV+ amniotic fluid specimens (21/ 43; 49%), and (3) any prenatally collected ZIKV+ specimens (20/41; 49%), though these comparisons did not

reach statistical significance (.05<*P*<.1). Zika-associated birth defects were statistically significantly more commonly among pregnancies in women with ZIKV+ prenatal amniotic fluid specimens (19/32; 59%) and among women with ZIKV infection and symptom onset before or during the first trimester (13/ 22; 59%), than those with all ZIKV— specimens (*P*<.05; Table 3). However, there was no statistical difference in prevalence of Zika-associated birth defects between pregnancies with ZIKV+ prenatal amniotic fluid specimens (19/ 32; 59%) and those with other ZIKV+ specimen types exclusively (10/23; 43%; *P*>.05). Among women with any ZIKV+ amniotic fluid specimens, C_T values were similar among pregnancies with and without Zika-associated birth defects (data not shown).

Women with 2 amniotic fluid specimens

Eight women had 2 amniotic fluid specimens that were tested for ZIKV; however, an amniotic fluid specimen collection date was missing for 2 of the women. Among the 6 women with amniotic fluid collection dates, 1 woman had no laboratory evidence of ZIKV infection (Table 4). Among the 5 remaining women, all had an initial ZIKV+ amniotic fluid; 3 subsequent amniotic fluid specimens (collected 45—49 days later) were ZIKV— and 2 subsequent specimens (collected 51 and 131 days later) were ZIKV+. Of the 3 women with an initial ZIKV+ amniotic fluid specimen and a subsequent ZIKV— amniotic fluid specimen, 2 had infants with Zika-associated birth defects, and 1 did not. Of the 2 women with 2 ZIKV+ amniotic fluid specimens, 1 woman had an infant with Zika-associated birth defect, and the other did not.

Comment

Principal findings

This analysis presents one of the largest cohorts of pregnant women with amniotic fluid tested for ZIKV by rRT- PCR, with data collected via a national surveillance system during the height of the ZIKV outbreak. Although ZIKV testing of amniotic fluid identified some additional ZIKV infections that were not detected by other clinical specimens, testing of amniotic fluid did not identify all infections. Zika-associated birth defects were more common in pregnancies of women with prenatal detection of ZIKV in amniotic fluid than those with ZIKV— specimens but were similar to pregnancies in women with ZIKV that was detected in other specimen types exclusively.

Results in the context of other observations

In our analysis, some women transitioned from ZIKV+ to ZIKV— rRT-PCR results; ZIKV RNA was not detected in 11 of 19 amniotic fluid specimens that were collected prenatally or at delivery after a ZIKV+ serum specimen and 3 of 5 amniotic fluid specimens after a previous ZIKV+ amniotic fluid specimen. These findings suggest that maternal ZIKV RNA does not always persist in amniotic fluid, which is consistent with findings from serum and urine[15] and small case series of amniotic fluid.[22,26,36] Shaub et al[26] studied 6 women with ZIKV RNA that initially was detected in amniotic fluid and found that ZIKV tests of subsequent amniotic fluid specimens were negative in 3 women at 2—10 weeks after initial positive specimens. The authors credit these findings as either because of false negatives or

the fetal immune response's potential removal of ZIKV RNA particles from maternal-fetal circulation.

Amniocentesis is often performed to evaluate genetic and/or infectious causes of congenital anomalies. Our data add to the evidence base that ZIKV RNA can be cleared from amniotic fluid and demonstrate that clearance of ZIKV RNA does not necessarily result in the absence of birth defects. Most published studies assessed ZIKV testing of amniotic fluid among pregnancies with Zika-associated birth defects detected by prenatal imaging.[22,26,31] Data are limited regarding amniotic fluid testing of pregnant women with ZIKV infection without prenatally detected Zika- associated birth defects. Our results suggest that ZIKV RNA detection in amniotic fluid is not always associated with an infant/fetus experiencing Zika- associated birth defects. One-half of the women (22/43) with a ZIKV RT-PCR positive amniotic fluid specimen did not have an infant/fetus with evidence of Zika-associated birth defects; however, this analysis does not include follow up of children to assess for neurodevelopmental disabilities. In our analysis, 2 of 3 women with an initial ZIKV positive amniotic fluid specimen and a subsequent negative amniotic fluid specimen had an infant/fetus with evidence of a Zika-associated birth defect, and the third woman did not. These findings suggest that (1) ZIKV can be cleared from the amniotic fluid even when the infant/fetus has brain and/or eye defects potentially associated with ZIKV infection, (2) conversion from a positive to a negative amniotic fluid result does not indicate the absence of birth defects, and (3) repeated amniotic fluid sampling to monitor ZIKV infection is not warranted in pregnancies that are affected by maternal ZIKV, particularly given the risk of potential, albeit rare, complications that are associated with amniocentesis.

Clinical implications

Current guidance suggests that amniocentesis should be individualized because the absence of ZIKV RNA detection in amniotic fluid does not mean absence of fetal infection; however, if amniocentesis is performed as part ofclinical care, amniotic fluid ZIKV rRT-PCR testing should be performed potentially to provide additional evidence of ZIKV infection.[12] Our findings support these recommendations: among women with multiple specimen types tested (including amniotic fluid that was collected prenatally or at delivery) and ≥1 ZIKV+ specimen, almost one-half (29/68) had ZIKV— amniotic fluid and only other specimens that were ZIKV+; still, but almost one-quarter of women (15/68) received a confirmatory diagnosis of ZIKV infection by testing of amniotic fluid specimens alone. However, we would not suggest amniocentesis for the sole indication of detecting maternal ZIKV infection. For other congenital infections such as cytomegalovirus, sensitivity of amniotic fluid testing is highest when collected ≥20 weeks gestation and 6 weeks after cytomegalovirus infection, because sufficient time typically has elapsed for cytomegalovirus in fetal urine to accumulate in amniotic fluid.[37,38] Overall, our analysis indicates that testing of amniocentesis specimens that are obtained for other clinical reasons may provide additional confirmatory evidence of ZIKV infection during pregnancy, even if no previous laboratory evidence is available. Although amniocentesis would not be warranted for the sole purpose of establishing maternal ZIKV infection, testing amniotic fluid for ZIKV in specimens that are obtained for other reasons may result in a maternal diagnosis, which may prompt a change in prenatal and postnatal evaluation of the infant.

Research implications

Based on our findings, future research is needed in the following areas: (1) the optimal time to perform amniocentesis and its sensitivity to detect and diagnose congenital ZIKV infection, (2) longer- term outcomes among infants who are born to women with ZIKV+ amniotic fluid, and (3) ZIKV viral load variation by specimen types. We found a higher prevalence of Zika-associated birth defects among women with ZIKV RNA detected in prenatally collected amniotic fluid compared with women without laboratory evidence of ZIKV infection or women with ZIKV RNA that was detected in amniotic fluid collected on the day of delivery. However, the occurrence of Zika-associated birth defects was similar among women with ZIKV RNA detected only in specimen types exclusively. These findings are novel and bear further investigation and replication. Our analysis included only information on the birth hospitalization; further studies to assess for neurodevelopmental outcomes and longer- term follow up among infants who are born to mothers with ZIKV+ amniotic fluid are warranted. Last, our results suggest that ZIKV loads maybe higher in amniotic fluid than other specimens. Few previous studies have examined ZIKV viral load variations by specimen type; however, 2 small case reports also noted lower C_T values in amniotic fluid than serum.[39,40]

Strengths and limitations

Our analysis has several important limitations. Data were collected as part of routine national surveillance; decisions to collect specimens were solely those of the women and their healthcare providers. Colombian insurance covers ≥1 ultrasound scan per trimester of pregnancy (or per month, if ZIKV infection is suspected) and, if a birth defect is detected, an amniocentesis. Symptom onset date was based on self-report, and no data on specific symptoms were collected. Given the mild nature ofZIKV infection, symptom onset maybe subject to recall bias. In some instances, specimen collection dates were missing or implausible. Wherever possible, dates were confirmed through medical record review, although the high proportion of amniotic fluid specimens that were obtained on the day of delivery might reflect misclassification of specimen collection dates. Additionally, specimens that were collected during delivery are more at risk for cross-contamination, and no information was available as to the method of specimen collection. Our sample of women are not representative of all pregnant women because, in most cases, there was likely a clinical suspicion of a birth defect or other indication that prompted prenatal amniocentesis. This also meant that we were unable to estimate the sensitivity of amniotic fluid in diagnosing ZIKV infection. However, no analysis could ethically accomplish this, given the risks that are associated with amniocentesis. ZIKV detection was based on rRT-PCR tests, which have a low false-negative rate; however, some infections may still have been undetected.[41] Last, our data were insufficient to draw conclusions regarding the risk of congenital ZIKV infection or longer-term outcomes (eg, neurodevelopmental) because almost all infants had information available regarding only their birth hospitaliza- tions.[42] Still, the strengths of our analysis include the large sample size relative to other previous studies, inclusion of women with pregnancies that were unaffected by Zika-associated birth defects, and clinical review to apply a standard Zika-associated birth defect case definition.

Conclusions

Our analysis has clinical importance that indicates that testing of amniocentesis specimens that were obtained for other clinical reasons can provide additional confirmatory evidence of ZIKV infection during pregnancy, even in situations in which no previous laboratory evidence is available. Although we found that Zika- associated birth defects were more common among women with ZIKV RNA that was detected in prenatally collected amniotic fluid compared with women without laboratory evidence of ZIKV infection or women with ZIKV RNA detected in amniotic fluid that was collected on day of delivery, there was no difference compared with those women with other ZIKV+ specimen types. Thus, at present, it does not appear that we can rely on amniotic fluid testing above and beyond the testing of other specimens to identify infants with potential Zika-associated birth defects. The optimal time to perform amniocentesis and the sensitivity to detect and diagnose congenital ZIKV infection remains unknown. Healthcare providers should continue to discuss the risks and benefits of amniocentesis with their patients; at present, there is no clear evidence to suggest a need to change the current recommendations for amniocentesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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AJOG at a Glance

Why was this study conducted?

The clinical importance of Zika virus testing of amniotic fluid compared with other specimens and the association between the detection of Zika virus ribonucleic acid in amniotic fluid and birth defects are unclear.

Key findings

Among 68 women with multiple specimen types tested and laboratory evidence of Zika virus infection, amniotic fluid identified 39 Zika virus infections (57%). Among 89 women with pregnancy outcome information, Zika-associated birth defects were significantly more common in pregnancies of women with prenatally collected Zika virus—positive amniotic fluid specimens (19/32 women; 59%) than with all Zika virus—negative specimens (6/23 women; 26%), but not different from those with other Zika virus—positive specimen types (10/23 women; 43%).

What does this add to what is known?

Testing of amniotic fluid can identify some, but not all, maternal Zika virus infections. Zika-associated birth defects were more common in pregnancies with Zika virus—positive prenatal amniotic fluid specimens than Zika virus—negative specimens.

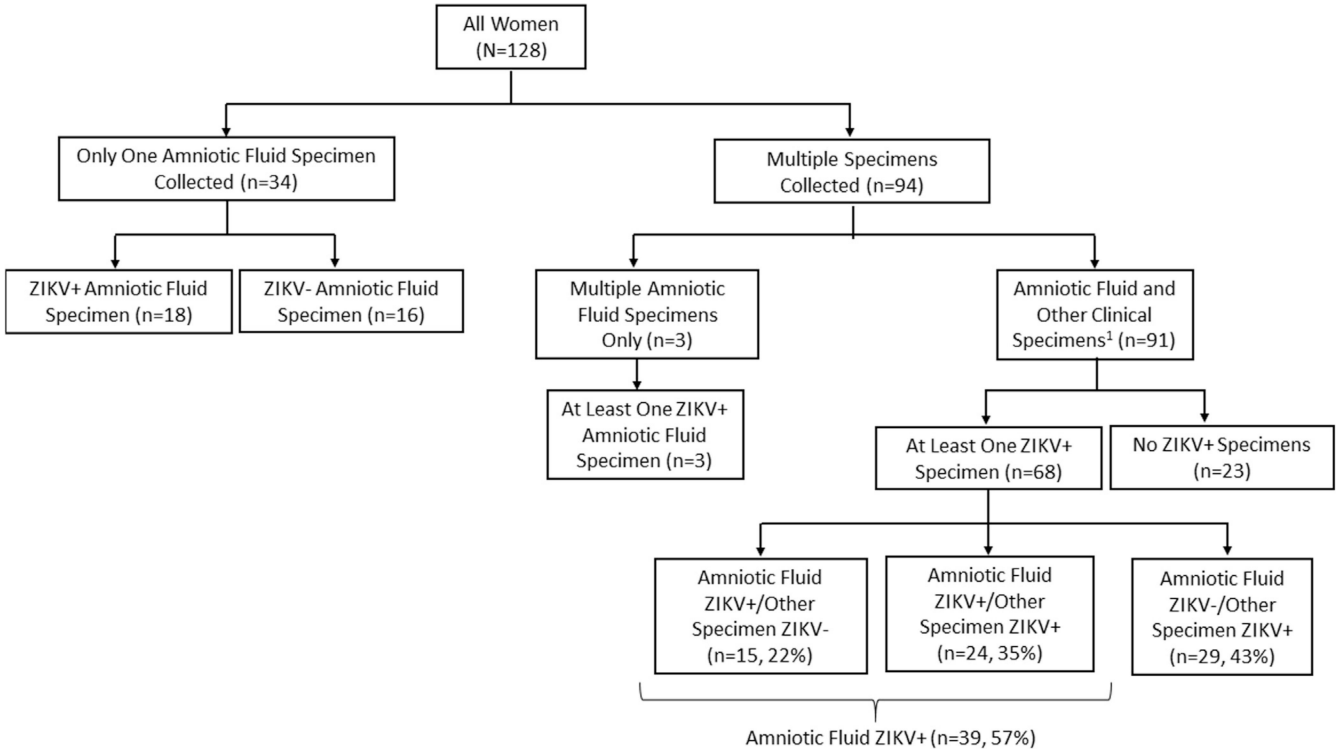

FIGURE.

Collection and Zika virus real-time reverse transcriptase-polymerase chain reaction test results for amniotic fluid specimens relative to other specimens (N=128)

TABLE 1

Characteristics of women with amniotic fluid specimens collected for Zika virus real-time reverse transcriptase–polymerase chain reaction testing (N = 128)

Characteristic	N(%)
Maternal	
Maternal age, y	
<20	19(15)
20–24	32 (25)
25–29	38 (30)
30–34	18(14)
≥35	21 (16)
Onset of symptoms	
Before/during 1st trimester[a]	31 (24)
2nd Trimester	29 (23)
3rd Trimester	31 (24)
At delivery/end of pregnancy	10(8)
Unknown[b]	27 (21)
Outcome of pregnancy	
Liveborn infant[c]	87 (68)
Pregnancy loss	23 (18)
Unknown	18(14)
Clinical specimen	
AnyZIKV + specimen (n=128)	89 (70)
Timing of first ZIKV + specimen	
Before/during 1st trimester	11 (9)
2nd Trimester	23 (18)
3rd Trimester	18(14)
At de livery/end of pregnancy	30 (23)
Unknown	7(5)

Characteristic	N(%)
Not applicable (no ZIKV + specimens)	39 (30)
Amniotic fluid (n=128)[d]	
ZIKV+	60 (47)
Timing of sample collection (any result)	
1st Trimester	0
2nd Trimester	30 (23)
3rd Trimester	45 (35)
At delivery/end of pregnancy	42 (33)
Unknown	11 (9)
Maternal serum (n=63)[e]	
ZIKV+	22 (35)
Timing of sample collection (any result)	
1st Trimester	14(22)
2nd Trimester	15(24)
3rd Trimester	6(10)
At delivery/end of pregnancy	25 (40)
Unknown	3(5)
Other specimens	
ZIKV+	
Umbilical cord tissue (n=50)[f]	29 (58)
Placenta (n=39)[f]	27 (69)
Umbilical cord blood (n=31)[f]	11 (35)

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ZIKV+, Zika virus real-time reverse transcriptase–polymerase chain reaction positive specimens.

[a]Two women had Zika virus symptom onset at 2 and 3 weeks before estimated date of conception; 29 women had Zika virus symptom during the first trimester of pregnancy

[b]Symptom onset date was available, but date of birth was missing for 8 women

[c]Includes 6 pregnancies that resulted in neonatal death

[d] Eight women had a second amniotic fluid specimen tested for Zika virus (3 during the 3rd trimester of pregnancy; 4 at delivery, and 1 with missing specimen collection date)

[e] Eight women had a second serum sample tested for Zika virus, all of which were collected on day of delivery

[f] All specimens were collected on day of delivery.

TABLE 2

Timing and Zika virus real-time reverse transcriptase-polymerase chain reaction test results of amniotic fluid specimen collection relative to other clinical specimens (N=68[a])

	Timing of amniotic fluid collection and Zika virus real-time reverse transcriptase-polymerase chain reaction test result							
	Any time		Amniotic fluid before other specimen		Amniotic fluid on same day as other specimen[b]		Amniotic fluid after other specimen[b]	
Other clinical specimens	**ZIKV+**	**ZIKV–**	**ZIKV+**	**ZIKV-**	**ZIKV+**	**ZIKV–**	**ZIKV+**	**ZIKV–**
Maternal serum (n=50)								
ZIKV+	11[c]	11	0[c]	0	3[c]	0	8[c]	11
ZIKV–	20	8[d]	5	2[d]	9	4[d]	6	2[d]
Umbilical cord tissue (n=38)								
ZIKV+	16[c]	13	13[c]	7	3[c]	6	r̂[e]	_[e]
ZIKV–	8	1[d]	6	0[d]	2	1[d]	r̂[e]	_[e]
Placenta (n=31)								
ZIKV+	12[c]	15	11[c]	7	1[c]	8	_[e]	_[e]
ZIKV–	4	0[d]	4	0[d]	0	0[d]	_[e]	_[e]
Umbilical cord blood (n=21)								
ZIKV+	3[c]	8	0[c]	4	3[c]	4	_[e]	_[e]
ZIKV–	4	6[d]	3	1[d]	1	5[d]	_[e]	_[e]

ZIKV–, Zika virus real-time reverse transcriptase-polymerase chain reaction negative specimens; *ZIKV+*, Zika virus real-time reverse transcriptase-polymerase chain reaction positive specimens

[a]Restricted to women with amniotic fluid and at least 1 additional clinical specimen collected and at least 1 ZIKV+ specimen (Figure)

[b]May include amniotic fluid specimens collected on date of delivery

[c] Represents concordant positive Zika virus real-time reverse transcriptase-polymerase chain reaction test results between amniotic fluid specimen and other clinical specimen

[d] Represents concordant negative Zika virus real-time reverse transcriptase-polymerase chain reaction test results between amniotic fluid specimen and other clinical specimen

[e] Indicates no samples were collected at that time period. Other cells represent discordant Zika virus real-time reverse transcriptase-polymerase chain reaction test results

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TABLE 3

Number and proportion of pregnancies with and without evidence of a Zika virus associated birth defect[a] (N=89)

	Zika-associated birth defects[a]		No Zika-associated birth defects[a]					
Characteristic	**n**	**%**	**n**	**%**	**Total, n**	***P*value[b]**	***P*value[b]**	***P*value[b]**
All pregnancies	37	42	52	58	89	—	—	
Pregnancy outcome								
Pregnancy losses	15	65	8	35	23	.01[c]	—	
Live birth	22	33	44	67	66	Reference[d]	—	
ZIKV+ specimens								
At least 1	31	47	35	53	66	.08	—	
No ZIKV+ specimens	6	26	17	74	23	Reference[d]	—	
ZIKV+ specimen type								
Amniotic fluid	21	49	22	51	43	.07	.68	
Other specimen type(s) only	10	43	13	57	23	.22	Reference[d]	
No ZIKV+ specimens	6	26	17	74	23	Reference[d]	—	
Timing of ZIKV+ specimen collection								
Prenatal ly	20	49	21	51	41	.08	.71	
At delivery	11	44	14	56	25	.19	Reference[d]	
No ZIKV+ specimens	6	26	17	74	23	Reference[d]	—	
Timing of ZIKV+ amniotic fluid specimen(s)								

Characteristic	Zika-associated birth defects[a] n	%	No Zika-associated birth defects[a] n	%	Total, n	Pvalue[b]	Pvalue[b]	Pvalue[b]
Prenatal amniotic fluid	19	59	13	41	32	.01[c]	.24	.03[c]
Amniotic fluid at delivery	2	18	9	82	11	1.00	.25	Reference[d]
Other specimen only (at any time)	10	43	13	57	23	.22	Reference[d]	—
No ZIKV+ specimens	6	26	17	74	23	Reference[d]	—	—
Timing of symptom onset if any ZIKV+ specimen(s)								
Before/during 1st trimester	13	59	9	41	22	.03[c]	.18	
2nd Trimester	8	50	8	50	16	.13	.47	
3rd Trimester	5	33	10	67	15	.72	Reference[d]	
At delivery/end of pregnancy	1	17	5	83	6	1.00		
Unknown/missing	4	57	3	43	7	.18	—	
No ZIKV+ specimens	6	26	17	74	23	Reference[d]	—	

ZIKV+=Zika virus real-time reverse transcriptase-polymerase chain reaction positive specimens.

[a]Defined as brain abnormalities (with or without microcephaly) or eye abnormalities identified prenatally (for pregnancy losses) or confirmed postnatally (for live births

[b]Chi-square (or Fisher's exact chi-square for small sample sizes) probability value for comparison between specified row and Reference category

[c]$P > .05$

[d]Comparison group for chi-square or Fisher's exact (if any cell ≤ 5) tests.

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TABLE 4

Characteristics of women with 2 amniotic fluid specimens that were tested for Zika virus (N=8)

Pregnant woman	Laboratory evidence of Zika virus infection (from any specimen)	Pregnancy trimester				Pregnancy outcome	Zika virus–associated birth defects
		Earliest ZIKV+ specimen	Symptom onset	First amniotic fluid specimen[a]	Second amniotic fluid specimen[a]		
1	Yes	1st	Unknown	3rd (+)	Delivery (+)	Live birth	Yes
2	Yes	2nd	Unknown	2nd (+)	3rd (+)	Live birth	No
3	Yes	2nd	1st	2nd (+)	Delivery (-)	Live birth	Yes
4	Yes	2nd	2nd	3rd (+)	Delivery (-)	Live birth	No
5	Yes	3rd	1st	3rd (+)	Delivery (-)	Live birth	Yes
6	Yes	Unknown[b]	Unknown[c]	Unknown[c] (+)	Missing (-)	Missing	Unknown[c]
7	Yes	Unknown[b]	3rd	Missing (+)	3rd (-)	Missing	Unknown[c]
8	No	Unknown[b]	1st trimester	2nd (-)	3rd (-)	Missing	Unknown[c]

ZIKV+, Zika virus real-time reverse transcriptase-polymerase chain reaction positive result.

[a]Zika virus real-time reverse transcriptase-polymerase chain reaction result

[b]Not calculable because of missing specimen collection date or end of pregnancy date

[c]Not assessable because of missing pregnancy outcome information.

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