The Role of Amniocentesis in the Diagnosis of Congenital Zika Syndrome

Jose Paulo Pereira Jr., MD[1]*;Melanie M. Maykin, MD[2]*; Zilton Vasconcelos, PhD[1]; Elyzabeth Avvad-Portari, MD, PhD[1]; Andrea A. Zin, MD, PhD[1]; Irena Tsui, MD[3]; Patricia Brasil, MD, PhD[4]; Karin Nielsen-Saines, MD[5]; Maria E. Moreira, MD, PhD[1]; Stephanie L. Gaw, MD, PhD[2]

*JPP, Jr and MMM contributed equally to the manuscript

[1] Instituto Nacional de Saúde da Mulher, da Criança e do Adolescente Fernandes Figueira– Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

[2] Division of Maternal-Fetal Medicine, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, San Francisco, California, USA

[3] Jules Stein Eye Institute, Retina Division, University of California, Los Angeles, Los Angeles, California, USA

[4] Laboratorio de Doenças Febris Agudas, Instituto de Infectologia Evandro Chagas–Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

[5] Division of Pediatric Infectious Diseases, Department of Pediatrics, University of California, Los Angeles, Los Angeles, California, USA

Corresponding Author: Stephanie L. Gaw, MD, PhD; Dept. of Obstetrics, Gynecology, and Reproductive Sciences, 513 Parnassus Ave, Box 0556, HSE16, San Francisco, CA, 94143, USA. Tel: 415-476-0535. Email: stephanie.gaw@ucsf.edu

Abstract

There is limited data on amniocentesis as a diagnostic tool for congenital Zika syndrome. Here we report on a prospective cohort of 16 women with suspected Zika virus infection in a highly endemic area, and discusss the role of amniocentesis in the prenatal diagnosis of fetal Zika infection.

Keywords: ZIKV, TORCH infection, perinatal infection, pregnancy, vertical transmission

The identification of Zika virus (ZIKV) from the amniotic fluid of two fetuses with severe central nervous system (CNS) abnormalities in 2016 was the first evidence of vertical transmission of ZIKV [1]. ZIKV has since been established as the etiology of congenital Zika syndrome (CZS), characterized in its most severe form by microcephaly and severe CNS malformations, with rates of adverse pregnancy outcome exceeding 40% in some studies[2]. Prenatal diagnosis of CZS informs management of the pregnancy and care of the ZIKV-exposed infant. However, the optimal method of prenatal screening and diagnosis of ZIKV infection remains undetermined. Testing is complicated by the asymptomatic course of most infections, the narrow window of viral RNA detection in maternal serum and/or urine, and limitations in maternal serologic testing due to delayed seroconversion, and false positive results from cross-reactivity with other flaviviruses [2,3]. Amniocentesis, the gold standard for prenatal diagnosis of vertical transmission in other infections, has not been routinely recommended in cases of suspected CZS, due to the invasive nature of the test (which carries a small risk of pregnancy complications), the limited data available to guide the timing of testing and interpretation of results, and limited therapeutic options [3]. Available data on the performance of ZIKV detection in amniotic fluid has been limited to isolated case reports and small case series of confirmed CZS that have reported a total of 27 cases [1,4-11].

Here, we report on a prospective cohort of patients referred for suspected ZIKV infection during the 2015-2016 Rio de Janeiro epidemic. Instituto Fernandes Figueira of Fiocruz Institute is the Ministry of Health referral center for Rio de Janeiro state, and provides comprehensive prenatal, obstetric, and postnatal care for high risk pregnancies complicated by complex fetal diagnoses. In this cohort, 16 women underwent amniocentesis after being referred for clinical suspicion of ZIKV infection due to abnormal CNS ultrasound findings suggestive of ZIKV or another TORCH infection. Some of the women reported symptoms compatible with ZIKV infection earlier in pregnancy and had exceeded the 3 week

window for blood and urine testing for ZIKV RNA at our institution, or had negative maternal testing earlier in pregnancy.

Upon referral, all women underwent a comprehensive prenatal ultrasound evaluation, performed by perinatologists certified by the Brazilian College of Radiology and the Brazilian Federation of Societies of Gynecology and Obstetrics. Amniotic fluid was collected, and patients were offered fetal karyotype analysis, as well as nucleic acid testing for ZIKV, cytomegalovirus (CMV), and toxoplasmosis. Patients also underwent maternal blood testing for HIV with viral load, toxoplasmosis IgM/IgG, rubella IgM/IgG, and VDRL for syphilis. After delivery, neonates underwent a comprehensive evaluation, including neonatal examination, ophthalmologic exam, and neuroimaging with transfontanelle ultrasound and/or head CT. Placenta, infant urine, serum, and/or cerebrospinal fluid were tested for ZIKV by RT-PCR as available.

From January to October 2016, 16 women underwent amniocentesis for possible ZIKV infection. All had fetuses exhibiting CNS abnormalities on ultrasound. **(Table)**. Twelve of 16 (75%) women reported symptoms consistent with ZIKV infection. Of the patients who had symptoms, all but one reported symptoms in the first trimester of pregnancy. Case 11 tested positive for ZIKV in urine at 12 weeks gestation. Two patients (Cases 1 and 8) had negative ZIKV testing earlier in pregnancy for suspected infection. The most common ultrasound abnormalities were: ventriculomegaly (12/16, 75%), CNS calcifications (8/16, 50%), microcephaly (defined as <2 SD below the mean; 6/16, 38%), and fetal growth restriction (defined as <10%ile; 10/16, 63%). Detailed description of ultrasound results and relevant prenatal testing are shown in the **Supplemental Table.**

The median gestational age at the time of amniocentesis was 26 weeks (range 18 to 34). Eight mothers reported symptoms of ZIKV infection. For symptomatic cases, the median elapsed time from suspected infection to amniocentesis was 16.5 weeks (range 10 to 27). There were 7 cases of ZIKV detected by RT-PCR in amniotic fluid, ranging from 10 to 19 weeks after suspected infection. There were two other cases of TORCH infection (CMV and toxoplasmosis, respectively), with amniocentesis detecting congenital infection in 56% of pregnancies. Eight patients consented for karyotype testing, of which one case confirmed trisomy 18.

In this cohort of fetuses with CNS abnormalities, amniocentesis detected 7/9 (78%) of CZS cases. Two additional cases were diagnosed through postnatal evaluation and testing of the neonate: Case 4 by placenta and fetal cord blood, and Case 11 by placenta, neonatal blood, and PCR of cerebrospinal fluid. Of the two CZS cases that were not detected on amniocentesis, the elapsed times from suspected infection to amniotic fluid testing were 27 weeks and >17 weeks. There were three patients who were found to be positive for CZS by amniocentesis but had PCR-negative postnatal samples. These included: Case 5 (negative neonatal blood, urine, placenta, and cerebrospinal fluid), Case 9 (negative neonatal blood and cord blood), and Case 15 (negative cord blood, urine, placenta, and cerebrospinal fluid). Detailed results of postnatal outcomes (evaluation, neuroimaging, and relevant tests) are reported in the Supplemental Table. Overall, there were 2 cases of stillbirth (13%) and 4 cases of neonatal death (25%), with a perinatal/ postnatal death rate of 38% in this high-risk cohort.

Discussion

To our knowledge, this is the first prospective cohort examining the role of amniocentesis as a diagnostic tool in cases of suspected ZIKV infection. In our cohort of 16 fetuses with CNS abnormalities on ultrasound during the 2016 ZIKV epidemic in Rio de Janeiro, there were 9 confirmed cases of CZS. Amniocentesis had a ZIKV detection rate of 63% (7/9).

The majority of cases were likely infected in the first trimester based on reported symptoms, and only two patients had maternal ZIKV testing by RT-PCR at the time of symptoms—all with negative results. All patients were referred for CNS abnormalities on ultrasound during the epidemic, where there was high risk of exposure to ZIKV and a high potential for CZS. Amniocentesis for genetics or suspected TORCH infection is not commonly performed in Brazil, as it is not routinely accepted by patients due to fear of pregnancy loss or other complications, such as infection or premature rupture of membranes, and the lack of pregnancy options.

As of July 2018, review of the literature found a total of 27 cases reported on the use amniocentesis for the diagnosis of ZIKV infection. However, we found no prospective reports, and determination of the sensitivity and specificity of ZIKV PCR as a diagnostic tool could not be determined. Our results suggest that in the setting of ultrasound abnormalities and high risk of ZIKV exposure, amniocentesis is helpful as a diagnostic tool to aid in prenatal diagnosis, pregnancy care, and optimization of pediatric support. The optimal time to perform amniocentesis remains to be determined. Meaney-Delman *et al.* detected ZIKV in amniotic fluid as early as 3 weeks after infection [12]. The window for molecular detection of ZIKV is longer than the usual time of active viral shedding in maternal blood or urine, which is between 1-4 weeks in pregnant women, although prolonged viremia is possible [2]. We successfully detected

ZIKV RNA in amniotic fluid between 10 and 19 weeks after suspected infection, which is consistent with other reports.

Importantly, a negative result on amniocentesis does not rule out vertical transmission and CZS. Viral shedding in amniotic fluid may be transient, as demonstrated in two of our cases (Cases 4 and 11), as well as in other reports [7,8]. In cases of high suspicion for CZS, testing of the placenta and the infant after birth is a critical component of comprehensive evaluation, especially as knowledge of the full spectrum of findings in CZS continues to be elucidated.

One limitation of our study is generalizability to cases of suspected ZIKV infection and normal ultrasound. Prospective studies in this population are urgently needed. Additionally, this cohort does not address the timing of vertical transmission from mother to fetus or the timing of development of ultrasound findings, as the majority of women were not tested at the time of symptoms, did not undergo a standardized protocol for ultrasound screening, and were not referred until they had an abnormal ultrasound. Women in our cohort had very high risk pregnancies, with serious CNS fetal defects, leading to fetal or neonatal demise in almost 40% of cases (2 stillbirths due to Zika and toxoplasmosis, 2 of 4 neonatal deaths due to Zika or chromosomal abnormalities, and 2 others of unknown etiology). Furthermore, greater numbers of cases with varying degrees of fetal disease severity are needed to more accurately determine sensitivity and specificity, as well the the effect of timing with respect to infection.

This study supports the utility of amniotic fluid testing for the diagnosis of vertical transmission of ZIKV. A diagnostic result was determined in 63% of cases, all of whom presented outside the established window for maternal ZIKV testing, and three of which had PCR-negative postnatal testing. Confirmatory testing of CZS is important for prenatal counseling, informed decision-making for the pregnancy, as well as delivery planning and care of the affected infant.

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Conflict of Interest

All authors have no conflicts of interests to declare.

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Case	GA of infection‡	GA of amnio	Time after infection*	ZIKV PCR in AF	KT	TORCH	GA at birth†	BW	ZIKV PCR Postnatal	Final Diagnosis
Symptomatic										
1	12w (Neg)	25w3d	13w	Neg	-	**T. gondii**	25w4d†	862	Neg	**Toxoplasmosis** Stillbirth
2	11w	24w6d	13w	Neg	46XX	Neg	38w4d	2375	Neg	**Unknown etiology** SGA (3%)
3	8w	27w2d	19w	**Pos**	46XY	Neg	32w2d†	-	**Pos**	**ZIKV** Stillbirth
4	7w	34w3d	27w	Neg	46XY	Neg	40w1d	2562	**Pos**	**ZIKV** SGA (2%)
5	8w	18w6d	10w	**Pos**	46XY	Neg	38w3d	3410	Neg	**ZIKV**
6	5w	32w6d	27w	Neg	-	Neg	38w3d	1700	Neg	**Unknown etiology** SGA (<1%) Neonatal death
7	16w	32w1d	16w	**Pos**	-	Neg	40w2d	2020	**Pos**	**ZIKV** SGA (<1%) Neonatal death
8	12w (Neg)	25w5d	13w	Neg	46XY	**CMV**	37w5d	3360	Neg	**CMV**
9	10w	25w4d	15w	**Pos**	46XX	Neg	41w1d	2620	Neg	**ZIKV** SGA (2%)
10	7w	24w6d	17w	**Pos**	-	Neg	39w4d	2224	**Pos**	**ZIKV** SGA (<1%)
11	12w (Pos)	30w4d	18w	Neg	-	Neg	40w6d	2710	**Pos**	**ZIKV** SGA (2%)

12	<13w	24w5d	>11w	**Pos**	46XY	Neg	37w6d	1920	**Pos**	**ZIKV** SGA (<1%)
Asymptomatic										
13	-	31w6d	-	Neg	Neg	Neg	34w0d	1870	Neg	**Toxoplasmosis** (Infant T. gondii IgM+)
14	-	24w4d	-	Neg	47XX;T 18	Neg	30w2d	924	Neg	**Trisomy 18** Neonatal death
15	-	29w6d	-	**Pos**	-	Neg	39w1d	2400	Neg	**ZIKV** SGA (2%ile)
16	-	30w2d	-	Neg	-	Neg	-	-	-	**Unknown etiology** Neonatal death

Table. Main characteristics of cases that underwent amniocentesis for evaluation of suspected Zika virus (ZIKV) infection, Rio de Janeiro, Brazil, 2016.

Abbreviations: GA, gestational age; amnio, amniocentesis; AF, amniotic fluid; KT, karyotype; TORCH, results of PCR testing for cytomegalovirus (CMV), *Toxoplasma gondii* (T. gondii), and syphilis (serum VDRL); BW, birth weight (g); Postnatal, postnatal testing of placenta, cord blood, and/or neonatal serum, urine, or cerebrospinal fluid for ZIKV; pos, positive; neg, negative, SGA, small for gestational age (percentile).

‡ Result of maternal PCR if performed is in parenthesis.

* Time in weeks from gestational age at infection to amniocentesis

† stillbirth