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## Toxoplasmosis Molecular Testing Discrepancy in a Case of Asymptomatic Microcephaly

Luciana Friedrich<sup>1,2\*</sup>, Adrienne Rahde Bischoff<sup>3</sup>, Juliana Ritondale Sodr  de Castro<sup>2</sup>, J ssica Maria Gonalves Dias Cionek<sup>2</sup>, Juliane Zambrzycki<sup>2</sup>, Pauline Simas Machado<sup>2</sup> and Tamires Ferri Macedo<sup>2</sup>

<sup>1</sup>Neonatology Section, Hospital de Clinicas de Porto Alegre, Brazil

<sup>2</sup>Pediatric Department, Federal University of Rio Grande do Sul, Brazil

<sup>3</sup>Department of Pediatrics, Division of Neonatology, The Hospital for Sick Children and The University of Toronto, Canada

\*Corresponding author: Luciana Friedrich, Neonatology Section, Hospital de Clinicas de Porto Alegre, Brazil, Tel: 5551999534239; E-mail: lfriedrich@hcpa.edu.br

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### Abstract

**Introduction:** Microcephaly is a congenital anomaly associated with a wide range of etiological factors such as infections, medications/substances, genetic abnormalities and radiation. Environmental factors that are teratogenic must also be included in the differential diagnosis as common etiologies. In this report we describe a neonate with microcephaly whose molecular tests showed conflicting results in regards to the etiology of the condition.

**Case Report:** We describe a case of a term female neonate that was admitted for work up of microcephaly in a nursery in South Brazil. The maternal history was significant for HIV positivity, on irregular antiretroviral therapy, substance abuse (crack) and previous incomplete treatment for neurosyphilis. The initial investigations for the neonate were significant for toxoplasmosis IgG (45,000) and the cerebrospinal fluid analysis revealed a positive real time PCR for toxoplasmosis. Triple treatment for neurotoxoplasmosis was instituted (Sulfadiazine, Pyrimethamine and Folinic Acid). As complementary exams came all negative including cerebrospinal DNA sequencing for *Toxoplasma gondii* and the baby became constantly neutropenic, treatment was discontinued and the infant was sent home for follow-up with serologies and ophthalmological monitoring.

**Discussion:** This case described a new-born with microcephaly that was initially thought to be secondary to congenital neurotoxoplasmosis with a positive PCR in the CSF. However, the diagnosis was refuted with the absence of other clinical signs and once the DNA sequencing for *Toxoplasma gondii* was negative in the cerebrospinal fluid. The highlight of this report lies on the fact that two very accurate molecular tests showed divergent results. Although there were multiple risk factors for microcephaly in this patient, the definitive cause cannot be determined.

### Introduction

Microcephaly is a congenital anomaly associated with a wide range of etiological factors such as infections, medications/substances, genetic abnormalities and radiation [1]. Among the infectious agents, Zika virus infection is one of the latest epidemiologic highlights and has been associated with a large number of microcephaly cases in Brazil, particularly between 2015 and 2016 [2-4].

The Southern region, however, has a much lower incidence of notified cases during this period of time [5]. It is, therefore, imperative that other agents are considered, such as *Toxoplasma gondii* and human immunodeficiency virus (HIV), both endemic in this region [6]. Environmental factors that are teratogenic must also be included in the differential diagnosis as common etiologies. Molecular testing, such as Polymerase Chain Reaction (PCR) for toxoplasmosis and viral load for HIV are considered the gold standard diagnostic tests for such pathologies. In this report we describe a neonate with microcephaly whose molecular tests showed conflicting results in regards to the etiology of the condition.

### Case Report

We describe a case of a term female neonate that was admitted for work up of microcephaly in a nursery in South Brazil. Baby was born at 37 weeks of gestational age, birth weight 2520 grams (percentile 10-25<sup>th</sup>), head circumference 31 cm (percentile 3<sup>rd</sup>-10<sup>th</sup>), length 45 cm (percentile 3<sup>rd</sup>-10<sup>th</sup>). The maternal history was significant for HIV positivity, on irregular antiretroviral therapy, substance abuse (crack) and previous incomplete treatment for neurosyphilis. Mom's status was immune for rubella (IgG positive, IgM negative). As she did not have regular prenatal care, we did not have results of her viral load or CD4 values during pregnancy. At hospital admission, VDRL, serologies for Hepatitis B and C were all negative. At birth baby was started on nevirapine and zidovudine. The initial

investigations for the neonate included TORCH serologies which were significant for toxoplasmosis IgG (45,000) by two different techniques (Electro-chemiluminescence immunoassay and Enzyme-linked fluorescence assay) and negative toxoplasmosis IgM. The remainder of the serologies were negative, including a negative viral load for HIV, VRDL and PCR for Zika virus and Cytomegalovirus. PCR tests for Zika virus and Toxoplasma in the serum were not available at the hospital. The cerebrospinal fluid (CSF) analysis revealed protein 157 mg/dL, glucose 47 mg/dL, leukocytes 5/ $\mu$ L (neutrophils 52%, eosinophil's 2%, mononuclear 46%), red blood cells 29866/ $\mu$ L, negative culture, negative VDRL and a positive real time PCR for toxoplasmosis (Amplification for B1 gene-Platinum Sybr Green Q-PCR Supermix UDG-Invitrogen<sup>®</sup>, Extraction-QIAamp DNA Blood-QIAGEN<sup>®</sup>). Triple treatment for neurotoxoplasmosis was instituted on day of life 8 (Sulfadiazine, Pyrimethamine and Folinic Acid). Further investigations included a normal ophthalmological evaluation with indirect binocular ophthalmoscopy, head ultrasound and brain magnetic resonance imaging. The remainder of the physical exam, including a thorough neurological examination, was unremarkable apart from the microcephaly. The new-born routine screening test was also normal (tested for cystic fibrosis, congenital hypothyroidism, hemoglobinopathy, phenylketonuria, biotinidase deficiency and congenital adrenal hyperplasia).

On day of life 15 the neonate was noted to be neutropenic (700/ $\text{mm}^3$ ). Zidovudine was suspended for 72 hours and the dose of the Folinic Acid was increased, with resolution of the neutropenia in three days. On day of life 25 the complete blood count one more time showed neutropenia and zidovudine was discontinued permanently. There was persistent neutropenia during the following 30 days despite two trials of filgrastim. Given the absence of other findings compatible with toxoplasmosis apart from the positive PCR in the CSF, a Toxoplasma gondii DNA sequencing (Sanger sequencing<sup>®</sup>) in the CSF was performed and the results came back negative. Pyrimethamine and Sulfadiazine were discontinued with resolution of neutropenia within 3 days. The neonate remained asymptomatic throughout the hospital admission and was discharged home, with follow up planned for monthly toxoplasma serologies and indirect binocular ophthalmoscopy.

## Discussion

This case described a new-born with microcephaly that was initially thought to be secondary to congenital neurotoxoplasmosis with a positive PCR in the CSF. However, the diagnosis was refuted with the absence of other clinical signs and once the DNA sequencing for Toxoplasma gondii was negative in the CSF. The highlight of this report lies on the fact that two very accurate molecular tests showed divergent results.

The standard serological tests for toxoplasmosis are limited in the setting of congenital toxoplasmosis. The serology may be accurate to show acute infection in the mother, during the pregnancy, but IgG serology does not imply that the infection has been transmitted to the new-born. In those cases, PCR is the most common method used to identify the parasite [7]. The specificity and positive predictive factor are described as being higher than 99% [8]. We have previously shown in a cohort of 24

patients with congenital toxoplasmosis that microcephaly was present in 21% of the sample, but the most common features were retinochoroiditis (present in 54%), intracranial calcifications (37.5%) and altered CSF (37.5%) [9]. Although this patient presented with hyperproteinorrachia, the initial CSF sample was bloody and the cell count was low (<20/ $\text{mm}^3$ ). There were no intracranial calcifications or retinochoroiditis. The positive PCR in the first CSF sample could potentially be due to blood contamination, secondary to a positive PCR in the blood and not in the CSF. It was thought that congenital toxoplasmosis was an unlikely diagnosis in the absence of other key features, but this remains questionable and makes follow-up even more important.

In regards to the differential diagnosis of microcephaly, multiple factors must be taken into consideration. Within the congenital infections category, the mother was HIV positive and had a history of incomplete treatment for neurosyphilis. Apart from the previously discussed aspects related to congenital toxoplasmosis, HIV viral load, PCR for Zika virus and VDRL (plasma and CSF) were negative in this patient. It is important to mention that the ideal method for diagnosing Zika virus infection would have been serology, but this was not available. The PCR for Zika virus is known to be positive for a short period of time, therefore its negativity does not exclude the diagnosis [10]. However, in the absence of compatible epidemiology, other symptoms or dysmorphisms of the Congenital Zika virus Syndrome, this differential becomes unlikely.

The history of maternal substance abuse is another important feature. Crack is a free base form of cocaine that is usually smoked and it is highly addictive. Prenatal cocaine exposure disturbs GABA and dopamine development [11]. It has been associated with microcephaly and altered mental performance [11-12]. Chromosomal abnormalities are unlikely given the absence of dysmorphisms or symptoms in this case. Other possible differential diagnosis include placental insufficiency, maternal hypothyroidism, maternal malnutrition, maternal abdominal injury, etc. [13].

In summary, although there were multiple risk factors for microcephaly in this patient, the definitive cause cannot be determined. The initial diagnosis of congenital toxoplasmosis done by CSF PCR in this neonate was refuted given the negative CSF DNA sequencing and absence of other key signs and symptoms of the disease. The CSF PCR is considered the gold standard, and it is interesting to notice the divergent results [8,14]. Follow up is extremely important not only to trend results of toxoplasmosis IgG and repeated screening for retinochoroiditis, but as well as arranging appropriate interventions for the possible neurodevelopmental abnormalities that may arise in the setting of a microcephalic infant.

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