Travel Medicine and Infectious Disease 23 (2018) 14–20

Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevier.com/locate/tmaid

Congenital microcephaly: A diagnostic challenge during Zika epidemics

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ARTICLE INFO

Keywords:
Microcephaly
Zika
TORCH
Pregnancy infections
Latin America

ABSTRACT

The multiple, wide and diverse etiologies of congenital microcephaly are complex and multifactorial. Recent advances in genetic testing have improved understanding of novel genetic causes of congenital microcephaly. The recent Zika virus (ZIKV) epidemic in Latin America has highlighted the need for a better understanding of the underlying pathological mechanisms of microcephaly including both infectious and non-infectious causes. The diagnostic approach to microcephaly needs to include potential infectious and genetic etiologies, as well as environmental *in-utero* exposures such as alcohol, toxins, and medications. Emerging genetic alterations linked to microcephaly include abnormal mitotic microtubule spindle structure and abnormal function of centrosomes. We discuss the diagnostic challenge of congenital microcephaly in the context of understanding the links with ZIKV emergence as a new etiological factor involved in this birth defect.

1. Introduction

As of February 2018, ZIKV infections had been documented in 85 countries and territories, 49 of which are in the Americas, including Brazil, Colombia, Mexico, Guatemala, Honduras, El Salvador, Panama, Dominican Republic, Puerto Rico, Guadeloupe, Barbados, Ecuador, Venezuela, Surinam, Guyana, French Guyana, Bolivia, Paraguay, Costa Rica, Nicaragua and Peru [1]. To fully understand the impact of this emerging pathogen in the pediatric population, a comprehensive understanding on other causes of primary microcephaly affecting neonates (infectious and non-infectious) is highly relevant.

Although strong epidemiological evidence suggests viral circulation of Zika virus (ZIKV) in Brazil since 2013 [2], the onset to epidemic proportions of cases in Latin America since 2015 has triggered concerns due to a simultaneous increase in the reporting of congenital microcephaly cases.

It is clear now that this arboviral infection has been associated with an increased incidence of microcephaly in fetuses born to infected mothers [3]. Specifically, an increase in reported cases of congenital microcephaly observed during the last months of 2015 in Brazil have raised concerns in neighboring countries known to have circulation of this mosquito-borne pathogen.

The following paper will discuss a clinical approach to microcephaly in the Americas, with an emphasis on the diagnostic process for infectious etiologies from the clinician's perspective in the context of the emergence of congenital Zika virus syndrome.

1.1. Defining microcephaly

A measurement of head circumference (HC) (also called occipitofrontal circumference [OFC]), is determined by placing a measuring tape (with cm and mm scale) around the head to include the widest part of the forehead and the most prominent part of the occipital area to arrive at the largest possible measurement. According to this,

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https://doi.org/10.1016/j.tmaid.2018.02.002
Received 2 December 2017; Received in revised form 2 February 2018; Accepted 13 February 2018
Available online 19 February 2018
1477-8939/

Fig. 1. Cases of microcephaly of Colombia (photos taken by Jorge L. Alvarado-Socarras).

microcephaly is defined as a head size that is 2 standard deviations (SD) below the mean, based on age and sex, according WHO guidelines. Alternatively, Barkovich defines OFC as more than 3 SDs below the mean [4] (Figs. 1 and 2). If we are to follow the first definition mentioned above, 2.3% of neonates in the Americas would fall in the range as microcephalic where as severe microcephaly (considered as an OFC $\leq$3 SD at birth) would be expected in up to 0.1% of children assuming a normal distribution, which agrees with the published estimate of 0.14% of neonates [5]. WHO defines microcephaly as a HC below 2 standard deviations on the reference curves measured within the first 24 life hours. But, for full term neonates (> 37 weeks) the cut-off value is 31.5 cm and 31.9 cm for girls and boys respectively [6]. The definition of microcephaly (Figs. 1 and 2) used becomes particularly relevant considering that a value below the cut-off does not necessarily imply evidence of clinical neurologic or developmental impairment but may simply represent the low end of the population distribution. However, other factors can be adjusted and taken into consideration in these definitions, as for example the prematurity and parental head circumference [7]. In general, severity is related to prognosis.

1.2. Potential pitfalls in the measurement of head circumference

Measurement of the HC is an important parameter in the pediatric population and a series of measurements over time are generally regarded as more instructive than a single measurement. However, there are pitfalls in the interpretation of abnormal head circumference at birth [8]. The measured size of the head in comparison with age-related norms is used to determine the definition of macrocephaly or microcephaly and this is used as a preliminary screen for conditions associated with neurologic impairment. Nevertheless, the major issue now is how to define microcephaly when more than one criterion and reference patterns would be applicable in different populations and clinical scenarios [8].

Microcephaly is a clinical and anthropometrical sign, which can potentially signal an abnormality in brain growth and development with a reported incidence ranging from 1:6200 to 1:8500 [8]. However, it's true incidence may be confounded by differences in measurement and reporting, varying in different geographical settings [9].

1.3. Classification of microcephaly

Several classifications of microcephaly have been adopted over time. Microcephaly can be considered isolated, or in association with other anomalies, (chromosomal or syndromic conditions), linked to other growth parameters (symmetric or asymmetric) or distinct etiologic determinants (genetic or environmental) [10,11]. The most frequently used classification relies on the timing of onset. Congenital microcephaly (also defined as primary microcephaly), is present at birth or by 36 weeks' gestation [9]. These terms do not imply a distinct etiology and can be seen with either genetic or environmental causes of neurodevelopmental impairment [12]. Secondary microcephaly refers to a failure of normal brain growth and change in measured head circumference after birth [10] and is usually due to a subsequent loss of dendritic connections [13]. Also, microcephaly has been traditionally categorized based on Giacomino's classification as: (1) Microcephalia Vera, where brain size remains small without any sign of injury or deformation; (2) Microcephalia Aspuria, in which some pathological changes and injury to the brain can be observed, and (3) Microcephalia Combinata, where a small brain size with evidence of injury are observed [14].

Neuroanatomic abnormalities frequently associated with microcephaly include holoprosencephaly, atelencephaly, lissencephaly, schizencephaly, polymicrogyria, macrogyria, and fetal brain disruption sequence [15,16]. It is important to mention that in microcephaly, although the brain is usually very small, -usually 3 standard deviations below the mean-its architecture can remain grossly normal with no link to other systemic anomalies. In addition, pregnancy, delivery and the postnatal period usually follow an uneventful course. Affected patients almost always have mental retardation but an otherwise unremarkable neurologic examination. A sloping forehead and prominent ears are usually the classic dysmorphic features seen in these cases.

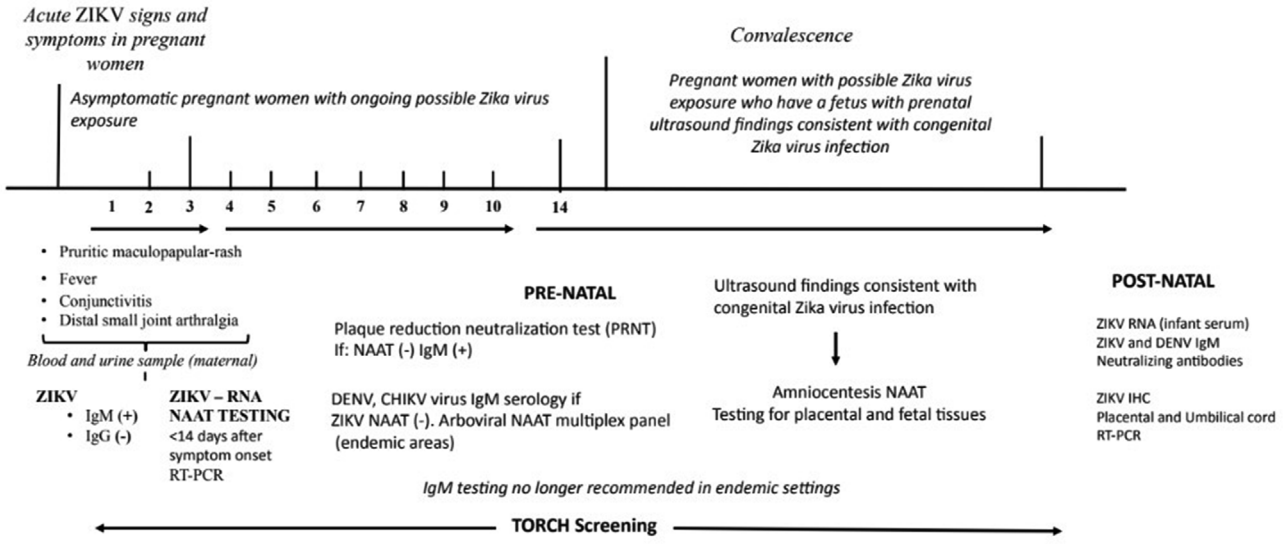

Fig. 2. Timeline: diagnostics of microcephaly.

Table 1
Risk factors for primary microcephaly (prenatal) [57 – 59].

Type	Risk factor by groups
Maternal	*Teratogens*
	Alcohol, hydantoin, radiation, tobacco, marijuana, cocaine, heroin, antineoplastics, antiepileptics, toluene
	Maternal untreated phenylketonuria
	Poorly controlled diabetes
	Pregnancy-Induced Hypertension
	Lead exposure
	Chronic renal failure
	Deficiencies
	Poorly controlled maternal hypothyroidism
	Folate deficiency
	Maternal malnutrition
	Placental Insufficiency
	Infectious
	TORCH: Toxoplasma, rubella, cytomegalovirus, herpes, syphilis.
	HIV, enterovirus
	Zika
Fetal	Neuropathic alterations
	Neural tube defect
	Holoprosencephaly
	Neuronal migration disorders
	Fetal brain disruption sequence
	Inborn errors of metabolism.

1.4. Non-infectious causes of microcephaly

Congenital microcephaly (Fig. 1) can be multifactorial, with a spectrum of clinical manifestations, ranging from conditions associated with severe impairment such as holoprosencephaly to milder cognitive or developmental delays or even normal development. Prenatal and perinatal environmental and/or genetic factors impacting on brain growth impairment should also be considered in developing our differential diagnostic workup in perspective with the current ZIKV epidemic (Table 1). In this context many causes non-infectious, briefly described below, causes should be assessed (see Table 2).

A history on exposures to known teratogens such as alcohol, hydantoin, radiation, maternal phenylketonuria and poorly controlled maternal diabetes, among others [11,17] should be carefully documented. Microcephaly may also be associated with other clinical findings that collectively comprise a genetic syndrome, such as the trisomies, Miller Dieker syndrome, Cornelia de Lange syndrome, Seckel syndrome, and Rubinstein Taybi syndrome [18]. Genetic variants associated with microcephaly include autosomal dominant, recessive, or X-linked conditions. Less frequently, a ring chromosome, mosaicism or and apparently balanced translocation may be identified. It is important to highlight that microcephaly has been reported in numerous syndromes with a large spectrum of clinical presentations and inheritance patterns, including various inborn errors of metabolism (aminoacidurias, organic acidurias, urea cycle disorders and certain storage diseases may be associated with microcephaly as well (10) with variability in the degree of associated psychomotor abnormalities [19]. In up to 41% of microcephalic neonates, a precise etiologic cause usually remains unknown [10].

Therefore, the evaluation of these patients usually requires array CGH based diagnosis, to define a phenotype associated with an etiologic diagnosis. Therefore, it becomes important to know whether a case falls into the congenital or postnatal microcephaly category; since several of these syndromes may present with microcephaly months, or even years later and thus should be excluded in the evaluation of congenital microcephaly [20].

Recently new emerging tools are proving helpful in the study of microcephaly. Next Generation Sequencing (NGS) also known as massive parallel sequencing, for example, allows interrogating many genes simultaneously with panels that include up to 12-to-50 target genes associated to microcephaly, thus, increasing diagnostics rates up to 8.5%. In addition, the advent of innovative platforms such as whole-exome sequencing, are proving to be a fundamental tool in diagnostics. However, due to its high costs, it is not recommended routinely in the assessment of these cases [19–21].

Table 2
Genetic causes of microcephaly [11,25,57 – 59]].

Type	Causes
Numerical Chromosomal aberrations:	Trisomy 13
	Trisomy 18
	Trisomy 21
	Others aneuploidies
Microdeletion and/or microduplication syndromes:	Deletion 4p Wolf – Hirschhorn
	Deletion 5p Cri-du-chat
	Deletion 22q11
	Deletion 17p13.3 Miller Dieker Syndrome
	Duplication 3q29
	Duplication 17q21.31
	Duplication Xq28
	Duplication 22q11
	Others deletion or duplication
Monogenic:	*Centrosome and Spindle Microtubule Defects Underlying Severe Congenital Microcephaly*:
	Autosomal Recessive microcephaly (MCPH1-16)
	Cortical Dysplasia, Complex, With Other Brain Malformations
	Defects in the Origin Recognition Complex Core:
	Meier–Gorlin Syndrome
	Defects in DNA Damage Response and DNA Repair Proteins:
	Seckel Syndrome
	Nijmegen Syndrome
	Bloom Syndrome
	Warsaw Syndrome
	Others:
	X-chromosomal microcephaly
	Aicardi–Goutieres syndrome
	Cockayne syndrome
	Cornelia de Lange syndrome
	Rubinstein–Taybi syndrome
	Feingold syndrome
	Rett syndrome, congenital
	Mowat–Wilson syndrome
	Smith–Lemli–Opitz syndrome
Metabolic Causes:	Serine biosynthesis disorder
	Sterol biosynthesis disorder
	Mitochondriopathy
	Congenital disorders of glycosylation syndrome

In addition, many environmental agents have been associated with microcephaly. Although research on this topic is still limited, in part because microcephaly is uncommon and causal associations are often difficult to establish by limitations of epidemiological methods [22], for the most part, intrauterine exposure to a potential teratogen is often recognized. Some case reports postulate causal relationships between a myriad of chemical agents and microcephaly, however evidence for most cases remain largely unsupported.

Yet, amongst the numerous environmental factors linked to microcephaly one has gained relevance in light of the ZIKV epidemics, the use of pesticides. The possible association between pesticides and microcephaly has remained a largely controversial topic. Microcephaly has been described in various studies involving agricultural human settlements [23]. Recently and during the current ZIKV epidemic, the widely used pesticide pyriproxyfen (a larvicide used in *Aedes aegypti* mosquitoes vector-control) was signaled as possible cause of microcephaly by Latin American environmentalists. However, a majority of scientists and research groups do not agree with this claim. Actually, a recent seminal work by Dzieciolowska et al., has demonstrated that even though pyriproxyfen is lethal at high doses, it does not affect zebrafish

Table 3
Infectious agents known to course or linked with the development of Microcephaly.

BACTERIAL
- *Borrelia burgdorferi* [34]
- *Chlamydia sp* [34]
- Group B *Streptococcus* [34]
- *Listeria monocytogenes* [34,35]
- *Neisseria gonorrhoeae* [34]
- *Treponema pallidum* [35]

FUNGAL
- *Candida sp* [34]

PARASITES
- *Toxoplasma gondii* [34]

VIRAL

Arenaviridae
Lymphocytic choriomeningitis virus [37]

Bunyaviridae: Bunyawera subgroup
Cache Valley (CV) [38]
Tensaw (TEN) [38]

Flaviviridae
Zika virus [39]
West Nile virus [40]

Herpesviridae
Herpes simplex 1 and 2 [39]
Cytomegalovirus [39]

Parvoviridae
Parvovirus B19 [35,36]

Picornaviridae
Enteroviruses [35]

Retroviridae
HIV/HTLV III [41]

Togaviridae
Rubella [39]
Chikungunya [42,43]

embryo development at the maximum recommended dose used in practice [24]. This observation has been further substantiated by current field research showing that there was no evidence of a correlation between Microcephaly and the use of pyriproxyfen in the municipalities Recife and Pernambuco during the Brazilian epidemic [25].

1.5. Infectious causes of microcephaly

Perinatal infections are among the top risk factors along with exposure to teratogens for developing of microcephaly (Fig. 2) [26]. A number of infections acquired *in utero* or during delivery have been linked to congenital defects (Fig. 2). Amongst these are bacterial and viral agents (Table 3). Typically, the infected newborn will show abnormal growth (intrauterine growth restriction, IUGR), developmental anomalies or multiple clinical and laboratory alterations. Routine screening for these infections, usually includes testing for syphilis, HIV, hepatitis B and toxoplasmosis, as well as other agents included in the so-called STORCH group. It is important to mention that within the spectrum of STORCH-related findings, microcephaly, seizures and intracranial calcifications [27] are of utmost importance in determining the differential diagnosis, including congenital ZIKV syndrome. Where possible, a thorough investigation for intrauterine infections linked to microcephaly (such as syphilis, toxoplasma, rubella, cytomegalovirus, herpes, HIV, hepatitis B (STORCH), malaria, parvovirus B19, *Trypanosoma cruzi*) should be considered (Fig. 2).

1.6. Congenital Zika virus syndrome

The ZIKV-associated cases of microcephaly reported by Kleber, and diagnosed during the first trimester of pregnancy, are by definition considered primary cases of microcephaly [28]. However, secondary microcephaly to ZIKV has been reported as well [29]. Additional studies by Zaria et al. in Brazil through 2016, revealed that microcephaly cases were best predicted in association to ZIKV during infections at week 17 of gestation on average (95% confidence interval of mean ± 0.11 weeks), or week 14 for suspected severe microcephaly cases (± 0.08 weeks). These findings are in general agreement with individual reports on the timing of reported maternal ZIKV symptoms that subsequently delivered infants with microcephaly [30]. However, ZIKV infections occurring later in pregnancy have also been linked to adverse outcomes, including fetal demise and intrauterine growth restriction [31].

Preliminary studies from Colombia suggest that maternal infection with ZIKV during the third trimester of pregnancy do not correlate with an increased incidence of structural abnormalities (such as microcephaly) in the fetus [32]. The risk for fetal microcephaly is highest for infections occurring on the first trimester, however cases of additional neurological impairments (non-microcephaly), have been reported to occur with infections during the last trimester of pregnancy [30,31] highlighting that the potential long-term neurodevelopmental impacts of maternal infection during pregnancy remain largely unknown.

Most infants with congenital infection remain asymptomatic or without apparent abnormalities at birth. Such is the case for ZIKV infection; although an increasing number of findings from both perinatal and congenital cases are being continuously reported [33]. To date, congenital ZIKV infection as mentioned earlier has been linked to a number of anomalies, mainly in the brain. Amongst these brain atrophy, calcifications, corpus callosum, and cerebellar vermis dysgenesia or agenesia, enlargement of the cisterna magna, lissencephaly, ventriculomegaly, and cerebellar hypoplasia have been observed [34,35].

Furthermore, ophthalmologic alterations such as cataracts, asymmetry of the orbital size, intraocular calcifications, macular atrophy, optic nerve hypoplasia, iris coloboma, and lens subluxation are increasingly being documented. In addition, other constitutional alterations like low birth-weight, excessive and redundant scalp skin, anasarca, polyhydramnios, and arthrogryposis [36] have been described.

In ZIKV specific associated neuroanatomic anomalies such as diffuse calcification of subcortical parenchyma and thalamus, ventriculomegaly, lissencephaly, and pachygyria have been distinctively highlighted [3,37], along with a constellation of particular neuropathological findings such as: gliosis, abnormal neuronal migration, dysmaturation of nerve cells, hypomyelination, loss of descending axons, and spinal motor neurons [35].

1.7. STORCH

Rubella, one of the etiological agents included in the classic STORCH complex, is rarely seen nowadays, with a very low incidence of congenital rubella syndrome, especially in those countries with robust vaccination schedules and coverage [38]. Classic infections included within the TORCH syndrome usually exhibit associated overlapping clinical findings as: hepatosplenomegaly and chorioretinitis [39]. Amongst the agents sharing greater similarities on the clinical arena with ZIKV are toxoplasmosis and cytomegalovirus (CMV). However, workup and testing must include an extended panel of agents including not only the but also: herpes, parvovirus, hepatitis B, HIV, Epstein-Barr (Fig. 2) [40].

Of note, other arboviruses have also been associated to adverse perinatal outcomes. Dengue for example has been linked to preterm delivery, low birth-weight, prematurity, acute fetal distress throughout delivery, and ultimately, fetal demise. West Nile Virus is known to cause chorioretinitis and focal cerebral loss. Rift Valley fever virus has been associated to an increased chance for miscarriages; as well as Chikungunya (CHIKV) where cases of encephalopathy have been described. In addition, the hemorrhagic fever are also a cause of congenital infection and late neurological development delay in infants [36,41].

Therefore, testing for such viruses should be included, particularly in the appropriate epidemiologic context (endemic areas, or returning travelers from endemic areas). Furthermore, co-circulation of arboviruses is becoming an increasing scenario in many tropical and subtropical areas of the world; thus, whenever ZIKV is suspected, consideration for other arboviruses should be taken into account as well.

Within this context, it is imperative to be familiar with another entity known as pseudo-STORCH. Pseudo-STORCH syndrome is a diagnosis of exclusion characterized by periventricular calcifications, associated to lesions in the basal ganglia, cerebellum and brainstem.

Microcephaly has been reported in up to 44% of these cases, with a variable degree of severity [42]. Pseudo-STORCH syndrome is a rare clinical entity, with an autosomal recessive pattern of inheritance, and which is characterized by the presence of microcephaly, intracranial calcifications, thrombocytopenia, mental retardation, seizures and hepatomegaly; with significant clinical overlap to the signs and symptoms that define congenital TORCH syndrome caused by *Toxoplasma gondii*, rubella virus, cytomegalovirus and herpes simplex virus. This syndrome cannot be diagnosed in clinical grounds alone, requiring an extensive workup based mainly on the exclusion of all infectious agents included within the TORCH syndrome list [43]. Therefore, testing for diverse infectious etiologies in such scenarios usually requires a comprehensive and multiplexing approach including serology, nucleic acid amplification tests (NAATs), cultures (urine, blood), placental and fetal pathology.

An important caveat is that screening for CMV during pregnancy is not included within routine workups. Routine screening for CMV infection during pregnancy, whether universal or targeted, is not recommended. The Society for Maternal Fetal Medicine does not recommend routine screening of all pregnant women for evidence of primary CMV infection at this time (grade 1B) [44]. Nevertheless, such situations should not apply in endemic areas of ZIKV, where testing should be mandatory, especially due to their overlapping clinical features. In addition, congenital cytomegalovirus (CMV) infection is a cause of significant neurologic morbidity and, to date there is a lack of comprehensive data on the prevalence of congenital disease in many parts of the world [45].

1.8. Prenatal and postnatal evaluation

Prenatally, microcephaly can be diagnosed by ultrasound examination (Fig. 2). The most common causes are *in utero* infections, followed less frequently by rare genetic syndromes and/or chromosomal anomalies [46]. The diagnosis is based in measurement of the head circumference, and suggested guidelines indicate a value of < 2 to < 3 SD below the mean for gestational age for diagnosis, however, this definition is not standardized due several variables such as sex and race [10].

Accurate estimation of gestational age (GA) is of utmost importance in order to plot appropriately fetal growth, in particular head circumference (HC) growth. To complete the evaluation, genetic testing, fetal brain magnetic resonance imaging (MRI) and testing for intrauterine infections should be performed. Genetic testing should be prompted in case of suspicion or associated risks and conditions such as parental consanguinity, family members with microcephaly and stigmata of autosomal dominant conditions that include microcephaly, abnormality of the central nervous system and other malformations suggesting chromosomal alterations, fetal microcephaly without clear cause or findings suggestive of intrauterine infection [10,46,47].

Therefore, prenatal screening of pregnant women with suspected ZIKV infection during pregnancy, must be as comprehensive as possible. Nowadays, there is an increasing body of knowledge suggesting that ZIKV can cross the placental barrier with reported cases of polymerase chain reaction (PCR) positivity in amniotic fluid of pregnant mothers with fetus's exhibiting structural brain abnormalities and microcephaly. Moreover, ZIKV has been isolated postmortem from the brain of a fetus with microcephaly [47,48] clearly depicting the selective neurotropism of this virus.

In context of a ZIKV epidemic, or in regions were the virus is endemic it is of highest importance to screen al pregnant mothers. Testing for ZIKV is possible in maternal serum by reverse transcription PCR (RT-PCR) or detection of ZIKV-specific IgM antibodies with subsequent confirmation by plaque reduction neutralization test (PRNT), which are not always available in endemic areas. Additional limitations include the narrow window of detection with RT-PCR testing, which can detect ZIKV only during acute infection (5 days) [49]. However, recent findings suggest the occurrence of prolonged ZIKV RNA detection in serum, which can persist in pregnant women for up to 46 days after the onset of symptoms, and even after 53 days' post-exposure in asymptomatic mothers [50].

Recently, the Pan American Health Organization (PAHO-WHO) and the Centers for Disease Control and Prevention (CDC) have updated their interim guidance on Zika management during pregnancy; this, in light of the falling prevalence of cases and as endemicity level equalizes across the world. Even though case definition remains the same, current recommendations urge that pregnant women with recent Zika virus exposure and symptoms of Zika should undergo Zika virus nucleic acid amplification test (NAAT) of serum and urine and IgM testing as soon as possible, through 12 weeks after symptom onset. On the other hand, for pregnant women without symptoms but with ongoing possible exposure to Zika, IgM testing is no longer recommended routinely. However, Zika NAAT testing 3 times during pregnancy should still be performed [51].

In addition, for those pregnant women with recent possible Zika exposure whose fetus exhibit ultrasound findings suggestive of congenital Zika syndrome, maternal testing with NAAT and IgM should be performed [51]. Furthermore, a baseline imaging evaluation including accurate assignment of gestational age, baseline ultrasound scan and subsequent ultrasound scans with potential deviations from normal should be recorded. Ultrasound evaluation not only assesses head circumference, but also fetal brain anomalies such as intracranial calcifications and/or ventriculomegaly. If ultrasound assessment shows a size head of 2 SD below the expected mean for gestational age, the diagnosis of microcephaly is then confirmed [47].

Once the diagnosis of microcephaly has been made, and if a high degree of suspicion for ZIKV infection prevails, besides currently approved diagnostic methods, an amniocentesis may be considered. However, current data is limited to case reports and the sensitivity and specificity of this test for detecting congenital ZIKV infection is yet not clear. Thus, one should balance the risk/benefit for both fetus and mother if considering performing an amniocentesis for diagnostic purposes [47,52]. Fetal brain magnetic resonance imaging, remains an option which to detect potential abnormalities that may be missed on ultrasound imaging. Recently the CDC has updated its comprehensive strategy for testing placental and fetal tissues, which can be done for diagnostic purposes in particular scenarios, including women without a diagnosis of laboratory-confirmed infection and with a fetus or infant with possible Zika-associated birth defects [51].

As mentioned earlier, other infectious agents such as toxoplasmosis and cytomegalovirus should be screened for. Both of these are known to cause severe neurological damage, including microcephaly and calcifications [1,31]. In this scenario amniocentesis should be considered. For toxoplasmosis, prenatal diagnosis is based in the assessment of the amniotic fluid. Amniotic fluid PCR exhibits sensitivity between 65 and 92% and specificity close to 100% [53]. In the case of cytomegalovirus, diagnosis during pregnancy is performed mainly by serology whenever there is clinical suspicion or ultrasonographic evidence suggestive for CMV infection. Once maternal infection has been confirmed, fetal screening should be performed. Even though there are several methods for evaluating fetal infection, the most reliable is PCR (sensitivity between 90 and 98% and specificity of 92–98%). Infections during the first trimester have greater severity, in a similar fashion to ZIKV infection. Therefore, ultrasound monitoring should be strict. The most relevant ultrasound findings in this setting are oligohydramnios or polyhydramnios, hydrops fetalis, intrauterine growth restriction, hepatosplenomegaly, intrahepatic calcifications, increased intestinal echogenicity, microcephaly, ventricular dilatation, cortical atrophy and intracranial calcifications [54]. It seems that the combination of maternal immunoglobulin M (IgM), immunoglobulin G (IgG) avidity index (AI) and fetal ultrasonography, can enable of detect infected fetuses having severe sequelae [55].

Postnatally, standardized HC measurements should be undertaken

and plotted on standards that consider GA at birth and sex. The use of a single cut-off regardless of GA is not recommended [47].

Assessment of microcephaly cases during ZIKV epidemics must be managed by an interdisciplinary team. A first step should include ZIKV infection confirmation on the mother and ultrasound screening for fetal abnormalities suggestive of infection. As mentioned earlier, in light of the ongoing ZIKV epidemic in Latin America and its clear association with neurological manifestations in the fetus, the CDC has issued guidelines for the evaluation and testing of infants with suspicion for possible congenital Zika Virus infection. These guidelines provide recommendations on the management of infants with prenatal diagnosis of microcephaly or intracranial calcifications; as well as recommendations on how to approach those cases born to mothers who were potentially infected with Zika Virus during pregnancy. However, as cases continue to drop, and prevalence keeps declining into the endemic channel, certain changes in the diagnostic approach should be considered. For example, because IgM can persist for months after infection IgM levels cannot reliably determine whether an infection occurred during the current pregnancy; thus, clinicians should no longer routinely recommend IgM testing to make confident decisions [51].

In sum, every neonate with microcephaly, born to mothers potentially infected with ZIKV during pregnancy could be tested through an enzyme-linked immunosorbent assay (ELISA) to detect anti-Zika virus IgM antibodies [56]; however, because the Zika virus IgM ELISA can provide false-positive results due to presence of cross-reacting IgM antibodies against other related flaviviruses, or previous exposure a plaque reduction neutralization test (PRNT) should be performed in order to measure virus-specific neutralizing antibody titers [57]. For these purposes an initial sample should be collected either from the umbilical cord or directly from the infant within 2 days of birth [57].

Unfortunately, a great limitation for a strict commitment to such guidelines and an accurate workup of cases, lays on the unavailability of diagnostic resources especially in developing countries currently undergoing large outbreaks; such is the case for Colombia and Venezuela. Other diagnostic methods include histopathological assessment of placenta and umbilical cord, as well as urine and cerebrospinal fluid analysis. Ultimately, and because of the difficulties posed by serological testing, RT-PCR remains the gold-standard confirmatory test for diagnosis. Furthermore, all ancillary tests should be accompanied by a thorough anthropometric evaluation including length, weight, and a precise assessment of gestational age (Ballard scale). A detailed clinical examination should focus on presence of specific phenotypic traits (dysmorphic features), megalies and full review of systems. Further testing such as cranial ultrasound, as well as auditory and visual evoked potential is of great complement to evaluate functional disability. Additional testing to rule out other congenital infections such as syphilis, toxoplasmosis, rubella, cytomegalovirus infection, lymphocytic choriomeningitis virus infection, and herpes simplex virus infections must be performed. In the appropriate epidemiological context, the possibility of co-circulation with other arbovirus, such as dengue and CHIKV should be taken into account [58,59].

Neuroimaging has lately gained relevance and is considered useful in further resolving evidence on structural causes in the evaluation of a child with microcephaly. MRI may be better than CT, because of its better performance in determining abnormalities such as migrational disorders, callosal malformations, structural alterations in the posterior fossa, and disorders of myelination. MRI studies be used to detect malformations associated to genetic conditions, which may be responsible for some cases of microcephaly (15–50%) [60]. Ultrasound may perhaps be performed if the fontanelle is of a sufficient size [61–63].

2. Conclusions

The diagnostic challenge posed by a growing number of etiological agents related to microcephaly and other related birth defects is highly relevant and calls for a multidisciplinary approach in its assessment and management. ZIKV has emerged as an important neurotropic pathogen within the list of agents related to microcephaly. Microcephaly itself has multiple long-term consequences in public health, particularly related to neurological disability. Considered the first cause of acquired microcephaly, infectious diseases multiplex screening is of pivotal importance to proceed for a proper antenatal screening in pregnant women living or returning from ZIKV or other arboviruses endemic areas. Early identification of microcephaly can be a critical first step in identifying disorders, leading to referral to specialists and, as needed, provision of family-centered early intervention services, amongst other multiple implications.

Competing interests

There is no conflict of interest.

Acknowledgments

The authors would like to thank Sonia Dickson for her critical and English review of the manuscript.

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