

# Tropical Infections of the CNS: A worldwide problem

Maria Teresa Acosta<sup>1</sup> , Arushi Gahlot Saini<sup>2</sup> , Douglas Postels<sup>3</sup> , Marco T. Medina<sup>4</sup>, Alfredo Cerisola<sup>5</sup> , Pratibha Singhi<sup>6</sup> 

<sup>1</sup>National Human Genome Research Institute National Institutes of Health Clinical Center Building 10, Room 3 I485 NE, Bethesda, MD 20892-3717, USA

<sup>2</sup>Department of Pediatrics, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India- 160012

<sup>3</sup>Department of Neurology, George Washington University/Children's National Medical Center, 111 Michigan Ave NW, Washington DC 20010

<sup>4</sup>National Autonomous University of Honduras, Centro de Investigaciones Cerebrales, Torre Medica, Tepeyac, Colonia Tepeyac, Tegucigalpa, Honduras

<sup>5</sup>Department of Neurology, School of Medicine, University of the Republic, Uruguay, Parva Domus 2388 / 101 – CP 11300

<sup>6</sup>Pediatric Neurology and Neurodevelopment, Medanta, The Medicity, Gurgaon, Haryana, India

Corresponding author: Maria Teresa Acosta; acostam@nih.gov

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## Abstract

Neuroinfectious diseases continue to have a profound public health impact, particularly in resource-limited settings. Here we summarize the new research into pathophysiology, diagnosis, and treatment modalities for some of the most frequent and lethal infections affecting the central nervous system, including tuberculosis, malaria, and arboviral infections. Implementation of clinical trials targeting neuroinfectious diseases in resource-limited settings has unique challenges not found when identical research is performed in resource-rich areas. Travel, communications, and technology have improved the mobility of populations worldwide. Immigration and increased international travel make it likely that clinicians worldwide will see patients affected by infectious diseases such as malaria, tuberculosis, zika, dengue, chikungunya, and Ebola. Such infections may have devastating consequences for both the individual and the society, particularly if clinicians are not familiar with disease presentation and treatment.

**Keywords:** CNS infection; malaria; tuberculosis; zika; dengue; chikungunya; clinical trials

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## Introduction

Central nervous system (CNS) infections are a global problem with profound public health impact, contributing to both the mortality and neurological morbidity in survivors. The Global Burden of Disease network estimated that in the year 2010, of all deaths in children 1–4 years old, meningitis and encephalitis caused 4.2%, acquired immunodeficiency syndrome 5%, malaria 21%, and tuberculosis 1.3% [1]. Causal pathogens vary by geographic regions, vaccination coverage, route of pathogen acquisition, as well as the patient's age and co-morbidities [2, 3]. Though the estimates of disease incidence and prevalence are publicly available, the actual burden of CNS infections in low and middle-income countries (LMICs) may be underestimated due to the relative lack of surveillance systems and diagnostic modalities [4].

Travel and improvements in communications and technology have improved the worldwide mobility of populations. Infectious diseases previously limited to specific geographic areas are

no longer limited to the places where prevalence is highest. With international travel, patients affected by infectious diseases such as malaria, tuberculosis, zika, dengue, chikungunya and Ebola can be encountered anywhere in the world. These infections may have devastating consequences for both the individual and society, especially if clinicians are unfamiliar with disease manifestations and treatment.

## CNS tuberculosis: recent concepts in diagnosis and treatment

According to 2018 World Health Organisation (WHO) estimates, there are one million children worldwide who are younger than 15 years old with tuberculosis; nearly one-fourth die annually. CNS tuberculosis, including tuberculous meningitis (TBM) and tuberculomas, accounts for 5–10% of all clinical presentations in children. Children with TBM have a 19% risk of death (95% confidence interval [CI] 14.0–26.1); the probabil-

ity of survival without neurological sequelae is 36.7% (95% CI 27.9–46.4) [5]. Delay in treatment initiation is the highest risk factor for mortality. Unfortunately, due to a lack of universally available and sensitive diagnostic tests, an early diagnosis is frequently challenging. Even if a diagnosis is made in a timely fashion, the duration of anti-tuberculous therapy (ATT) for children with TBM is controversial.

The diagnosis of TBM relies on the clinical, cerebrospinal fluid (CSF) and neuroimaging features. The presence of non-CNS tuberculosis in the patient or family members is an essential epidemiological risk factor for the diagnosis. Fever is the most common sign (60–95%), followed by seizures (50%) and cranial nerve deficits (30–50%) [6]. Making a clinical diagnosis may be challenging, as young children frequently present with non-specific symptoms of poor weight gain, low-grade fever and irritability. Though the average duration between symptom onset and initial presentation at a healthcare facility for those with TBM is 5–30 days, many children do not arrive until comatose or with other classic neurological findings [7].

Neuroimaging, when available, is especially important for early diagnosis. Though computed tomography is more common in resource-poor settings, magnetic resonance imaging (MRI) is more sensitive (Figure 1) [8]. In addition to neuroimaging, other supporting or diagnostic laboratory tests may help the clinician to establish a diagnosis (Table 1). The poor sensitivity of nucleic acid amplification-based testing is believed to be due to the paucibacillary nature of TBM in children, the presence of amplification inhibitors in the CSF, and challenges with cell lysis in biological specimens due to the impermeable cell wall of *M. tuberculosis* [9].

**Figure 1.** Contrast-enhanced magnetic resonance imaging of the brain in a child with tuberculous meningitis showing exudates in the basal cisterns and leptomeningeal enhancement in the axial section.



A recent meta-analysis concluded that nucleic acid amplification-based tests' diagnostic accuracy is currently insufficient for them to replace culture as the only diagnostic

modality [10]. A Cochrane review showed a pooled sensitivity of 54% and specificity of 94% for Xpert MTB/RIF (*Mycobacterium tuberculosis*/rifampin) [11]. The authors concluded that in children with presumed TBM, treatment decisions should be based on the entirety of clinical information. Treatment initiation should not be withheld based solely on a pending or negative Xpert MTB/RIF result. WHO guidelines for tuberculosis state that commercial serodiagnostics should not be used in children suspected of active pulmonary or extrapulmonary TB, irrespective of their human immunodeficiency virus (HIV) status. Moreover, in LMICs, interferon-gamma release assays should not replace tuberculin skin testing to diagnose latent or active tuberculosis infection in children (irrespective of HIV status). Due to the high rates of coinfection, routine HIV testing should be offered to all patients with presumptive and diagnosed tuberculosis, including children [12].

Hydrocephalus and/or vascular strokes commonly complicate TBM. In 80% of survivors, communicating hydrocephalus is present, secondary to blockage of CSF resorption in the basal cisterns from inflammatory exudates. Medical and surgical management of secondary intracranial hypertension may include emergency ventricular tap or extra-ventricular drainage, or a more definitive CSF diversion method such as a ventriculoperitoneal shunt [13]. Strokes occur in 15–57% of paediatric TBM patients and are secondary to caseous meningoencephalitis and vasculitis, with or without superimposed vascular thrombosis. Basal ganglia infarction is common due to medial striate, thalamotuberal, and thalamic perforator involvement [14]. A systemic review of 546 patients with TBM evaluated the benefits of aspirin co-administration in the management of TBM [15]. Though the addition of aspirin to the ATT regimens did not significantly reduce mortality, adjunctive aspirin reduced the risk of new CNS infarctions compared to controls (RR = 0.52, 95% CI = 0.29–0.92) [15]. TBM may be complicated by vision loss secondary to optic atrophy, chiasmal tuberculoma, optico-chiasmal arachnoiditis, cortical blindness, or ethambutol toxicity [16, 17]. The spinal form of tuberculosis may manifest as tubercular arachnoiditis, myelitis, acute spinal cord or cauda equina syndromes, an intramedullary spinal tuberculoma, syringomyelia, tuberculous spondylitis (extradural) or Pott's disease [18, 19].

The treatment of paediatric CNS tuberculosis includes acute management of raised intracranial pressure, ATT, corticosteroids, anti-epileptic drugs, management of complications, and optimising nutrition and physiotherapy. The recommended ATT regimen consists of four drugs (isoniazid, rifampicin, pyrazinamide and ethambutol in fixed-dose combination) in the intensive phase for two months, followed by two drugs (isoniazid, rifampicin) in the maintenance phase for ten months. Higher doses of ATT and modified regimens have been recommended, but the duration of ATT must be at least 12 months [12, 20, 21]. For those with multi-drug resistant disease, the WHO has recently suggested consolidated guidelines for treatment [22]. A recent systematic review of treatment outcomes for children with multi-drug resistant tuberculosis determined a pooled estimate for treatment success of 82% (95% CI 72.5–90.8). When children are treated, outcomes are at least as good as those for adults

[23]. Research priorities moving forward should include determining optimal doses and duration of ATT in children with CNS tuberculosis, with or without multi-drug resistance.

HIV coinfection with TBM is associated with an earlier age at disease presentation, CSF neutrophil predominance, reduced meningeal enhancement, reduced odds of secondary hydrocephalus (believed to be secondary to a reduced inflammatory response from impaired cell-mediated immunity), diffuse brain atrophy, and a poorer overall prognosis [24, 25]. Immune reconstitution syndromes involving the CNS are common in children in whom antiretroviral therapy is initiated. The recovering immune system mounts an exuberant inflammatory response against mycobacterial antigens, and clinical symptoms usually begin within three weeks of starting antiretroviral therapy [25]. Affected patients undergo a clinical or radiological deterioration after an initial period of clinical improvement on ATT. The recommendation for prevention of TBM-IRIS (immune reconstitution inflammatory syndrome) is to withhold anti-retroviral therapy (ART) until after 4–8 weeks of anti-TB treatment. Management includes simultaneous treatment of TB and HIV, with the regimen determined by CD4 counts. Unfortunately, there is little evidence-based guidance for the management of paradoxical, tuberculosis-related immune reconstitution inflammatory syndromes in children. Corticosteroids are the mainstay of treatment [26]. Interruption of ART may be necessary in life-threatening cases. Anecdotally, thalidomide, mycophenolate, chloroquine and infliximab have been used. Human recombinant interleukin-1 receptor antagonists have been used as steroid-sparing therapy for life-threatening protracted paradoxical inflammation in HIV-associated tuberculosis [27].

In summary, TBM should be considered for any form of meningitis of more than five days duration in endemic settings. Newer molecular tests may aid early diagnosis, but neuroimaging is very helpful in LMICs in making an early diagnosis. At least one year of ATT is indicated in children with TBM, monitoring for complications and considering neuroimaging before stopping ATT.

**Cerebral malaria:** Recent concepts in diagnosis and treatment The WHO estimated 228 million cases and 405,000 deaths due to malaria worldwide in 2018. Children under five years of age constituted 67% of all malaria deaths. More than 90% of cases and deaths occurred in Africa [28]. Although clinical malaria has decreased by about 37% in the past 20 years, most cases still occur in children aged six months to six years old. Five species of *Plasmodium* infect humans through the bite of the infected female *Anopheles* mosquito—*Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium knowlesi*. Of these, *P. falciparum* is the most common species associated with cerebral malaria (CM). The female mosquito inoculates sporozoites into the human blood, which infect liver cells and subsequently erythrocytes. The erythrocytic stages cause clinical symptoms, while the liver stages are responsible for relapses (*P. vivax* and *P. ovale*). Sequestration of parasitised red blood cells in the deep vascular beds of the brain is

found in children dying after a clinical diagnosis of CM, and is thought to be the most important pathophysiological process leading to coma and death.

The WHO defines CM as impairment of consciousness (Blantyre coma scale less than three and more than one hour after a clinical seizure), the presence of asexual parasites in the peripheral blood, and the exclusion of other causes of coma [29]. Neurological features may include seizures or agitation [30]. A paradigm shift in the clinical diagnosis of CM was the identification of a malaria-specific retinopathy identified at the bedside with a combination of direct and indirect ophthalmoscopy. Features include whitening in the perimacular and peripheral retina, pale colour of retinal vessels, retinal haemorrhages, with or without papilloedema. Autopsy studies of children dying of CM who had malarial retinopathy (and were diagnosed with retinopathy-positive CM) revealed high levels of sequestration of parasitised erythrocytes in cerebral venules [31]. Retinopathy severity correlates with the degree of sequestration of infected red blood cells in the retina and the brain [32]. In endemic areas, a viral CNS infection may coexist in children with clinical CM and cause diagnostic confusion. A recent study of CSF from 111 children with clinical CM found viral co-infections in 18% of those who were retinopathy-positive, and in 25% of those who were retinopathy-negative [33]. Neither mortality nor neurological morbidity was associated with the presence of a virus (odds ratio = 0.276, 95% CI 0.05–1.36). The authors concluded that viral nucleic acid in the CSF of children with CM may be incidental bystanders, reactivating during acute malaria infection [33].

Observational studies on children with CM reveal that the neurological sequelae are common in survivors, most commonly hemiparesis, vision and hearing impairments, severe motor deficits, ataxia and learning disabilities. A recent meta-analysis of eight studies, enrolling a total of 2005 participants, determined that CM is associated with an increased risk of epilepsy (OR 4.68, 95% CI: 2.52–8.70), intellectual impairment (OR 4.72, 95% CI: 0.78–28.49), neuro disabilities (OR 16.16, 95% CI: 1.34–195.45), and behavioural disorder (OR 8.47, 95% CI: 2.75–26.04) [34]. A quarter of children have a cognitive impairment, which may be detected three to six years after recovery from the index illness. The cognitive impairment includes deficits in executive functioning, processing, memory, language and behaviour.

Currently available diagnostic tests for malaria include microscopy, rapid diagnostic tests, indirect fluorescent antibody test and PCR. Microscopy, using the Romanovsky (Giemsa) stain remains the gold standard for laboratory confirmation of malaria and allows species identification. Rapid diagnostic tests use point of care blood sampling to test for malaria-specific antigens. Negative rapid diagnostic tests should be confirmed with microscopy especially in non-immune travellers where parasitaemia may be extremely low at the time of symptom-onset. The indirect fluorescent antibody test is commonly used for screening of blood donors. PCR testing is currently used for research purposes and is frequently unavailable for routine clinical testing in malaria-endemic areas.

**Table 1.** Summary of the diagnostic tests utilised for tuberculosis and tuberculous meningitis [7, 8, 35].

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**Predictive clinical features of TBM in children**

- Duration of symptoms are more than five days: most predictive
  - Glasgow coma score is less than 15
  - Presence of focal neurological deficits
  - Neutrophil count is less than 50% of total white cells
- 

**Clinical stages of TBM**

Stage I: Lucidity, no focal neurological signs or evidence of hydrocephalus

Stage II: Lethargy, confusion, mild focal signs, such as cranial nerve palsies or hemiparesis

Stage III: Advanced illness with delirium, stupor, coma, seizures, multiple cranial nerve palsies, and/or dense hemiplegia

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**Extra-cranial markers of disease**

- Mantoux test: A positive test identifies exposure to tuberculosis and not active disease; sensitivity 61%, reduced to 34% with HIV coinfection
  - Radiological evidence of pulmonary tuberculosis is seen in 60% of children
  - History of contact with another tuberculosis patient is present in 30–50% of children
- 

**Cerebrospinal fluid examination**

- Appearance: transparent or mild xanthochromia
  - Total cell counts: 100 and 500 cells per  $\mu\text{l}$
  - Lymphocytic predominance (60–85% of cases)
  - Early neutrophilic predominance (30–75%)
  - CSF opening pressure: more than 25cm water (50% cases)
  - Protein: elevated (100–500 mg/dl)
  - Glucose: low (absolute value less than 45mg/dl), CSF: blood sugar ratio below 0.5
- 

**Microbiological assays**

- Smear microscopy: Ziehl Neelsen stain: rapid and economical, sensitivity 10–60%
  - Culture: Traditional LJ medium (turnaround time 28-50 days), shorter with Bactec (MGIT960, Radiometric 460) culture medium
- 

**Neuroimaging [35, 36, 30]**

- CT scan: most common neuroimaging modality in resource-limited settings
  - Most specific (100%): pre-contrast hyperdensity in basal cisterns
  - Most sensitive (89%): basilar enhancement
  - Combination of hydrocephalus, infarction, and basilar enhancement is 100% specific
  - Bilateral basal ganglia infarcts: highly suggestive of TBM
  - Contrast-enhanced MRI: more sensitive, delineates mild basal meningeal enhancement, tuberculomas, miliary leptomeningeal tubercles, opto-chiasmatic arachnoiditis, spinal TB, detection, and localisation of infarct
  - Single-photon emission computed tomography (SPECT): evaluates vasculitis due to TBM, reduced blood flow in affected regions of the brain
  - Fluorodeoxyglucose (FDG)-positron emission tomography (PET): increased uptake in focal brain lesions, specifically in the meninges and cerebellum
  - MR angiography: segmental narrowing and irregular beaded appearance, moyamoya syndrome
  - Transcranial doppler: real-time information about cerebral blood flow velocities
  - MR spectroscopy: prominent lipid peak (representing caseation), slightly increased choline (cellular component), or a reduced NAA and creatine peak (partial volume effect)
  - Contrast-enhanced FLAIR: for early detection of paradoxical tuberculoma
- 

**Molecular analysis of CSF [37, 38, 39, 40]**

- Nucleic acid amplification tests: sensitivity 56%, specificity 98%
  - Yield improved by physical methods of cell lysis, such as shock treatment (freezing and heating) or Triton X 100 treatment combined with DNA extraction procedures
  - Loop-mediated isothermal amplification (LAMP) assay is a single-tube technique for the amplification of DNA and a low-cost alternative to detect certain diseases: sensitivity 88%, specificity 80% for MTB
-

- IS6110 (sequences of mycobacterial genome) and Protein b (38 kDa protein product which is specific for MTB complex) -based multiplex polymerase chain reaction (PCR) test: rapid method, sensitivity 77.8 %, specificity 100%
- MTB cell-free DNA in CSF: rapid and accurate, sensitivity 56.5%
- Multiplex PCR: amplify several target genes simultaneously, sensitivity 85%–95%
- CSF Xpert MTB/RIF: test that simultaneously detects MTB by PCR and resistance to RIF in less than two hours
- Xpert Ultra: improved sensitivity but insufficient negative predictive value to exclude TBM
- Xpert MTB/XDR: detects resistance to both first-line and second-line drugs
- GeneXpert Omni: underdevelopment, portable point-of-care diagnostic test
- Truenat ® MTB, Truenat ® MTB Plus, and Truenat ® MTB-Rif Dx: Chip-based, micro real-time PCR assays for diagnosis of TB and rifampicin resistance

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#### Newer tests in the pipeline

- Next-generation sequencing

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#### Biochemical analysis

- CSF adenosine deaminase (ADA)
- Simple, inexpensive, rapid, with a sensitivity of 75–83%, specificity 89–93%
- Cut-off value of 10.5 iu/l
- Values from 1–4 u/l: sensitivity more than 93%, specificity less than 80%, helpful to exclude TBM, between 4 and 8 u/l: insufficient to confirm or exclude TBM, values more than 8 u/l: sensitivity less than 59%, specificity more than 96%<sup>36</sup>
- ESAT-6 immunostain frequently used in enzyme-linked immunospot assay for detection of active tuberculosis: sensitivity 75%, specificity 90%
- 16 kDa (HspX) antigen: is an immunodominant antigen that is recognised in cases with active tuberculosis by ELISA: sensitivity 43%, specificity 95%
- Lipoarabinomannan ELISA: is a direct antigen-capture ELISA based on the detection of mycobacterial lipoarabinomannan in unprocessed urine, sensitivity 64%, specificity 69%
- TB-ELISPOT: is a T-cell based assay for the detection of infection with MTB, sensitivity 71%, specificity 57%
- CSF interferon gamma release assays: sensitivity 59–84%, specificity 73–89%
- BacT/ALERT : BacT/Alert (Organon Teknika Corp., Durham, N.C.) is an automated microbial detection system based on the colorimetric detection of CO<sub>2</sub> produced by growing microorganisms, sensitivity 25%, specificity 100%
- CSF Antigen detection tests: sensitivity 35–95%, specificity 95–100%
- Tuberculostearic acid on GCMS): sensitivity 80–100%, specificity 80–100%
- Proteomic analysis of CSF: sensitivity 80-100%, specificity 80–100%

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TBM, tubercular meningitis; HIV, human immunodeficiency virus; CSF, cerebrospinal fluid; LJ, Lowenstein Jensen; CT, computed tomography; MTB/XDR, multidrug-resistant tuberculosis/extensively drug-resistant; GCMS, gas chromatography mass spectrometry; TB-ELISPOT or T-Spot, enzyme-linked immunospot assay for interferon- $\gamma$ ; ESAT-6, 6 kDa early secretory antigenic target produced by MTB; MTB, *Mycobacterium tuberculosis*

Anti-malarial therapies have undergone recent advancements, with a shift from quinine or quinidine to artemisinin-based medications. Artemisinins kill parasites in all life stages and is associated with more rapid parasite clearance times. Moreover, treatment with artesunate significantly reduces the risk of death, both in adults (RR 0.61, 95% CI 0.50–0.75) and children (RR 0.76, 95% CI 0.65–0.90), compared to intravenous quinine [41].

Seizures, both clinical and electrographic, are common in children with CM [42]. A previously completed clinical trial of intravenous adjunctive phenobarbitone (compared to placebo) produced a reduction in seizures but increased mortality [43]. A double-blinded, randomised trial of comparing a single intramuscular injection of fosphenytoin with placebo did not show any remarkable difference in preventing seizures and neurological sequelae in children with CM and acute coma [44]. A recently published phase I/II randomised clinical trial of enteral levetiracetam versus intravenous phenobarbital (the standard of care) for children with CM showed equal efficacy (time of electrographic seizures) of the interventions, but a more favourable safety profile for levetiracetam [45]. Pharmacokinetic parameters were comparable to studies of enteral levetiracetam performed in high-income countries (HICs) in children with non-malarial illnesses.

**Figure 2.** Plain magnetic resonance imaging (MRI) of the brain, sagittal section of the T1-weighted sequence in a child with cerebral malaria showing diffuse brain swelling and incipient uncal herniation.



Thus far, numerous interventional clinical trials of adjunctive therapies for children with CM have been performed with endpoints of death, disability, or both. None of these has shown a clinically meaningful improvement in outcome for participants randomised to the intervention, compared to controls [46]. Recent insights into CM pathogenesis have revealed novel therapeutic targets for rational design of potentially beneficial interventions. Diffuse brain swelling is common in children with CM and is strongly associated with death (Figure 2) [47]. Since many children dying of CM have a pure respiratory arrest, in-

creased intracranial pressure is likely leading to herniation syndromes, compression of the respiratory centres, and apnoea. Mechanical ventilation may help to preserve life while diffuse brain swelling diminishes. Intravenous hypertonic saline may work as an osmotic diuretic, directly decreasing brain swelling. An ongoing Phase III randomised clinical trial is evaluating the effectiveness of mechanical ventilation and hypertonic saline in Malawian children with cerebral malaria at high risk of death (NCT03300648). One-third of the participants will receive treatment as usual, one-third will receive treatment as usual as well as being placed on a mechanical ventilator, and one-third will receive treatment as usual plus intravenous hypertonic saline.

Of particular interest to paediatric neurologists are the neurological syndromes after the clearance of parasitaemia in malaria. These include acute demyelinating encephalomyelitis, post-malaria neurological syndrome (within two months of illness), cerebellar syndrome (onset three to four weeks after the febrile illness), encephalopathy, ataxia, opsoclonus, benign intracranial hypertension, Guillain-Barré syndrome, and myelitis.

Efforts to better understand CM pathogenesis must continue. A better understanding of the processes between the bite of an infective Anopheles mosquito and death or neurological disability may allow for the design of clinical trials to test interventions found to be important in disease pathogenesis. A recent meta-analysis highlighted the importance of timely treatment in preventing severe disease and deaths in malaria [48]. This underscores the need to have prompt access to first-line anti-malarial drugs and early referrals to higher centres for severe cases.

### Dengue, zika and chikungunya: acute neuroinfections

Around 3.3 billion people live in the tropics, which constitute half of the world's population [49, 50]. High temperatures and vector abundance make these areas a high-risk area for establishing and spreading arboviral diseases. In the last decades, due to global climate change, many parts of the world outside of tropical areas are becoming 'tropical regions' [49]. In tropical regions, over twenty neglected tropical diseases affect over one billion people each year. Many neglected tropical diseases, including dengue, chikungunya and zika, have neurologic consequences.

The term 'arbovirus' (ar, arthropod; bo, borne) includes several families of RNA viruses spread by arthropod vectors, most commonly mosquitoes, ticks and sand flies. The families of viruses included in the arbovirus group are Flaviviridae, Togaviridae, Bunyaviridae and Reoviridae [51]. Neurotropic arboviruses preferentially infect the neurons in the CNS and belong to several different RNA virus families. Clinically relevant neurotropic arboviruses include flaviviruses (West Nile, St. Louis encephalitis and Japanese encephalitis viruses), bunyaviruses (La Crosse and California encephalitis virus), and New World alphaviruses (eastern, western, and Venezuelan equine encephalitis viruses), and recently zika, dengue and chikungunya viruses [52].

**Table 2.** Common neurological complications of zika, dengue and chikungunya virus infection.

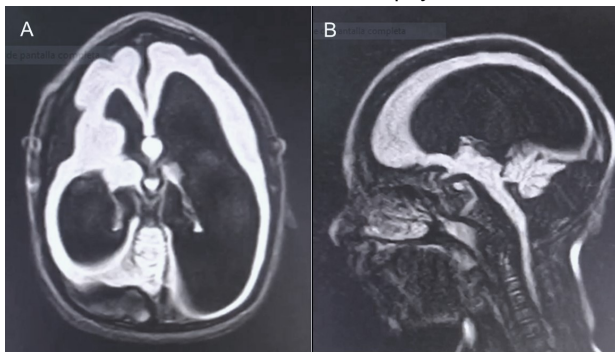
<b>Zika virus infection</b>
<ul style="list-style-type: none"> <li>• Congenital zika syndrome</li> <li>• Guillain-Barré syndrome</li> <li>• Sensory neuropathy</li> <li>• Encephalitis/meningoencephalitis</li> <li>• Inflammatory myelitis</li> <li>• Acute disseminated encephalomyelitis</li> <li>• Optic neuropathy</li> <li>• Seizures and epilepsy (epileptic spasms, focal and generalised onset motor seizures, myoclonus)</li> <li>• Vasculitis and childhood arterial ischaemic stroke</li> </ul>
<b>Chikungunya infection</b>
<ul style="list-style-type: none"> <li>• Meningoencephalitis</li> <li>• Myelitis</li> <li>• Guillian Barré syndrome</li> <li>• Seizures</li> <li>• Psychosis</li> <li>• Encephalo-myelo-radiculitis</li> <li>• Brain stem encephalitis</li> <li>• External ophthalmoplegia</li> <li>• Facial palsy, sensorineural deafness</li> <li>• Optic nerve involvement (papillitis, retrobulbar neuritis, neuroretinitis, optic neuritis)</li> </ul>
<b>Dengue virus infection</b>
<ul style="list-style-type: none"> <li>• Encephalitis</li> <li>• Encephalopathy</li> <li>• Meningitis</li> <li>• Stroke</li> <li>• Cerebellar syndrome</li> <li>• Transverse myelitis</li> <li>• Acute disseminated encephalomyelitis</li> <li>• Guillain-Barré syndrome</li> <li>• Myositis</li> <li>• Hypokalemic paralysis</li> <li>• Dengue-associated neuritis (such as brachial neuritis, long thoracic nerve palsy, phrenic nerve palsy, abducens nerve palsy and peripheral facial palsy)</li> </ul>

Arbovirus infections are associated with a wide spectrum of neurological manifestations. We review here zika, dengue and chikungunya infections, all of which have rapidly expanded their geographic distribution.

The zika virus is a single-stranded RNA Flavivirus that causes encephalitis and fetal microcephaly and is transmitted by the bite of *Aedes* mosquitoes. It was first isolated from a rhesus monkey in the Zika forest in Uganda in 1947 and from *Aedes africanus* mosquitoes in 1948 [53]. In 2007, a zika outbreak in the Yap Islands in the Federated States of Micronesia represented the first time the virus was detected outside Africa and Asia [54]. In February 2016, the WHO declared the zika virus infection a global public health emergency following reports of clusters of microcephaly and other neurological disorders in 2015 in Brazil [55, 56]. By 2017, the WHO estimated that nearly 100 million people were infected, including more than one million pregnant women in the Americas, suggesting that tens of thousands of children were at risk for the congenital ZIKV syndrome [57, 58].

The acute clinical syndrome resembles dengue or chikungunya. Over the years, zika virus infection has become neuroinvasive with reports of several neurological complications (Table 2) [59]. The underlying pathogenesis may include: direct infection of neural stem cells in the fetal brain; immune response against peripheral myelin and/or axonal components; infection of mature CNS neurons with ensuing cell death and pathogenic transcriptional dysregulation; endothelial injury; or a direct viral inflammatory process in the CNS [60, 61]. The initial description of the presumed congenital zika syndrome and congenital microcephaly from Brazil included a facial dysmorphism, cutis gyrata (scalp skin folds, secondary to continued skin growth concurrent with cerebral malformations), preservation of primitive reflexes, hypertonia, hyperreflexia, irritability, tremors, seizures, vision and hearing defects, intracranial calcifications, ventriculomegaly and/or lissencephaly (Figure 3) [56]. Symptomatic intrauterine infection may result in severe growth retardation, microcephaly, agyria, hydrocephalus, and multifocal dystrophic calcifications in the cortex and subcortical white matter, with associated cortical displacement and mild focal inflammation [62].

**Figure 3.** MRI in a Honduran girl of two years and nine months with congenital zika syndrome: T1-weighted (A) axial, and (B) sagittal sections showing a lissencephaly/pachygyria abnormality, ex-vacuo hydrocephalus, and diffuse brain stem and cerebellar atrophy.



Dengue is the most widespread arboviral disease, with an estimated 70–140 million cases occurring annually. There are four genetically and antigenically distinct serotypes of dengue. It is common to see concurrent or sequential infection of multiple serotypes. In 2009, WHO adjustments in the classification of the disease resulted in the recognition of two main disease manifestations: dengue fever and severe dengue [63]. Dengue virus infection causes an acute self-limited febrile syndrome characterised by headache, retro-orbital pain, rash, nausea, vomiting, diarrhoea, myalgia and arthralgia. In individuals with prior exposure to the dengue virus, re-exposure to a different serotype increases severe dengue risk [63]. Severe dengue (formerly called ‘dengue haemorrhagic fever’) is characterised by increased vascular permeability, thrombocytopenia, hemo-concentration, decreased effective circulating blood volume, and haemorrhagic manifestations. Neurological involvement is present in 4–20% of confirmed dengue cases [64]. High body temperature, elevated haematocrit, thrombocytopenia, rash and liver dysfunction are independent risk factors for neurological complications [65]. Neurological manifestations include encephalitis, meningitis, myelitis, and Guillain-Barré syndrome (Table 2). The underlying pathogenesis may include metabolic disturbances resulting in encephalopathy, direct viral invasion, and autoimmune reactions producing optic neuritis, encephalopathy, and Guillain-Barré syndrome [66].

Chikungunya virus is a togavirus that is transmitted to humans by the bite of *Aedes aegypti* or *Aedes albopictus* mosquitoes. Chikungunya infection was first detected in Southern Tanzania in 1952. The name ‘chikungunya’ in Swahili means ‘that which bends up’ and alludes to the classic clinical symptoms of fever and joint pain. Joint pain can persist for months to years after infection, causing patients to adopt a bent, stooping posture. The infection classically manifests with fever, rash and arthralgia. Though the virus is believed to be neurotropical, the neurological manifestations are poorly described (Table 2) [67]. Neonates with vertically acquired infections may have encephalopathy, white matter lesions, or cerebral oedema or haemorrhage [68].

Encephalitis is the most common neurological symptom and may co-occur within a few days of the onset of systemic symptoms. The virus induces morphometric and innate immune activation of astrocytes in the brain, thus causing them to express high levels of cytokines and chemokines in response to viral infection [69].

The zika, dengue and chikungunya arbovirus infections and their neurological consequences are a major global health problem. Diagnosis is based on clinical, epidemiological and laboratory criteria. Treatment is supportive. Immunologically-mediated disease manifestations are frequently treated with immunomodulatory agents (corticosteroids, intravenous immunoglobulin or plasmapheresis) though no evidence-based recommendations are available.



## Clinical trials in tropical infections: challenges and successes

Implementation of clinical trials evaluating interventions for tropical infections can be challenging but may significantly impact public health. Challenges to clinical trial implementation may include a lack of research infrastructure in LMICs where such diseases are prevalent. Fragile supply lines challenge equipment maintenance for clinical care and laboratory studies, mainly when these cross international borders.

With careful planning and development of mitigation strategies, these challenges can eventually be overcome. More challenging is cultural and linguistic barriers. Investigators from HICs may have significant differences from both the populations they are studying and the LMIC collaborators with whom they are working. This may lead to misunderstandings and, if not corrected, a breakdown in the collaborations necessary for clinical trial success. Although attempts to use the local language are often welcomed by local populations, incorrect word use may lead to unintentional offence [70]. Moreover, methods of handling conflict may differ significantly between investigators from HICs and LMICs. Pre-emptive discussion of possible points of disagreement may help avoid conflict. Verbalising expectations from all parties prevent misunderstandings.

Despite these challenges, implementing clinical trials in tropical infections may have significant benefits for HIC investigators and the populations they are studying. Co-investigators from HICs are typically self-selected with a commitment to social justice. LMIC co-investigators are equally self-selected for their work ethic and commitment to the populations with whom they live and work.

Performance of clinical trials in tropical infections may significantly impact populations living in areas where these diseases are found. Tropical infections are common and can lead to mortality (e.g., malaria, viral haemorrhagic fevers, trypanosomiasis) or morbidity (e.g., filariasis). Small effect sizes will have significant health impacts when prevalence is high. Despite the challenges of clinical trial implementation in some LMICs, investigating new therapeutic options for tropical infections is a significant health priority, conferring benefits on both an individual and population level.

### Competing interests

None.

### Author contributions

All authors have contributed equally to drafting the manuscript and revising it critically for important intellectual content and have given the version's final approval to be published.

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