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EDITOR'S COMMENT

THE FUTURE OF VIRAL INFECTIONS

Since the onset of the COVID-19 pandemic there is increasing scientific interest in animal viruses and their future cross-species transmission potential.

Although viruses are the most diverse and abundant of living organisms, we know less about their diversity, evolution, and cross-species transmission potential than other living organisms.[1]

Few animal species, mainly those located in the phylum Chordata have been surveyed for viruses, while surveillance among invertebrate species has mainly focussed on common disease vectors such as ticks and mosquitoes, both located in the phylum arthropoda, one of 21 invertebrate phyla. The recent advent of metagenomic next-generation sequencing has however begun to advance the study of the animal virome, revealing novel biodiversity insights. For example, while bats and rodents have been shown to harbour a wide range of coronaviruses, recent molecular explorations have documented coronaviruses in other vertebrates such as amphibians and fish.[1]

Large scale molecular studies, a feature of the scientific output arising from the current COVID-19 pandemic, have provided important information about the evolutionary trajectory of SARS-CoV-2. Despite having descended from a bat coronavirus, SARS-CoV-2 was immediately successful in humans because of efficient human-to-human transmission. The virus has started to adapt to its host through the evolution of a series of distinct variants capable of more efficient human-to-human transmission. The recently emergent Omicron variant provides possible clues to the future relationship of SARS-CoV-2 and its human host, being highly transmissible, exhibiting immune escape with loss of neutralisation activity when exposed to plasma from vaccinated individuals, and causing less severe disease than prior variants.[2]

It is estimated that approximately 10,000 viruses circulating in wild mammals have the capacity to infect humans. Zoonotic spill-over is predicted to increase substantially during the next 50 years. Robust quantitative modelling suggest that climate and land use changes caused by global warning will bring humans and wild mammals into closer proximity particularly in areas of high population density, creating many opportunities for cross-species virus transmission. However, it is unclear how this will influence human health.[3]

The response to the COVID-19 pandemic provides important lessons for future outbreak and pandemic preparedness. The rapid development of diagnostics, therapeutics, and vaccines, as well as epidemiological insights contributed substantially to the global response to the COVID-19 pandemic. The management of future outbreaks and pandemics will undoubtedly rely on contributions of the scientific community. However, the COVID-19 pandemic also taught us that resilient health systems, overcoming global and in-country inequities, community engagement and participation, robust social safety nets, and attention to communication, misinformation, community mistrust and scepticism, pandemic fatigue and mental health, and bioethics are important considerations.[4]

Brian Eley, editor

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SOCIETY NEWS

WSPID WEBINARS AND JOURNAL CLUB

In September 2020 WSPID launched its webinar series, one of several innovations aimed at strengthening infectious diseases educational activity, particularly between WSPID conferences. To date, ten webinars have been hosted addressing a range of topics, including the worldwide impact of COVID-19 in children under 18 years of age; managing children with HIV and TB in the shadow of a global pandemic; the changing epidemiology of respiratory viral infections in the COVID-19 pandemic; COVID-19 vaccines coming to light: current developments and near-future use and challenges; antimicrobial resistance in the COVID-19 pandemic and post-pandemic period; emerging issues in paediatric dengue; and the utility of syndromic testing for the diagnosis and management of meningitis/encephalitis. Thus far, the average live attendance has been a gratifying 521.

In April 2022, Young WSPID launched a global journal club with planned meetings every second month. The first meeting on 26 April 2022, was entitled “safety and efficacy of a typhoid conjugate vaccine in Malawian children” and focussed on a phase III clinical trial that was published in the New England Journal of Medicine in September 2021. The presenter was Dr Tinsae Alemayehu of Ethiopia. The second meeting on 28 June 2022 featured a Lancet publication entitled “antibiotics for lower respiratory tract infection in children presenting in primary care in England (ARTIC-PC): a double-blind, randomised, placebo-controlled trial”. This paper was presented by Dr Inês Silva Costa of Portugal. The average live attendance for these two meetings was 71.

Recordings of previous webinars and journal club meetings are archived on the WSPID Global E-learning portal at https://wspid.org/wspid-global-e-learning-portal/.

Information about forthcoming events may also be found on the WSPID Global E-learning portal. Please do view previous recordings and register for forthcoming events. Registration for access to the E-learning portal and future events is free of charge.

COMMENTARIES & REVIEWS

A REVIEW OF SEPTIC ARTHRITIS IN CHILDREN

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Abstract

Septic arthritis is a bacterial joint infection which most commonly occurs in children younger than five years of age. Diagnosing septic arthritis in children can be challenging as signs and symptoms can be non-specific. An accurate history, physical exam, laboratory investigations and imaging can contribute to the timely diagnosis of septic arthritis and limit chronic morbidity of joint dysfunction. The aim of this article is to review the epidemiology, clinical features, pathophysiology, differential diagnosis, special investigations, and management of septic arthritis in children.

Introduction

Septic arthritis (SA) is a bacterial synovial joint infection that most commonly occurs in young children with the highest incidence in those younger than five years of age.[1,2] This condition is an orthopaedic emergency and delay in the diagnosis and inappropriate management can lead to lifelong disability.[2] SA is typically a monoarticular condition with the most commonly affected joints being the knees, hips and ankles which account for up to 80% of the cases, but any joint can be affected.[3] A retrospective study done at Steve Biko Academic Hospital, Pretoria, South Africa between 2005 and 2009 described the phenotype of 44 children, less than 12 years of age with a suspected diagnosis of SA.[4] This study found that the knee was the most commonly affected joint followed by the hip, shoulder, elbow and ankles.[4]

It is well known that diagnosing SA in children can be challenging, as signs and symptoms overlap with other joint pathologies. This is especially true for neonates and infants in whom non-specific signs can include fever, refusal to feed, crying and limitation of limb movements. Older children typically present with joint immobility in association with fever, malaise and pain. Around 20% of children have a history of injury to the affected limb or a non-specific fall before presentation.[3]

An accurate history, physical exam, laboratory investigations and imaging can contribute to the timely diagnosis of septic arthritis. Management of SA includes drainage of the affected joint and appropriate antibiotics. The aim of this article is to describe recent developments in the diagnosis and management of SA.

Epidemiology and risk factors

Overall incidence of SA is 4 to 10 per 100 000 children in well-resourced countries and 1 in 5000 children in sub-Saharan Africa.[3,5–7] The incidence of SA in South Africa is unknown due to lack of literature regarding its epidemiology.
A systematic review by Gigante et al. described the following risk factors for developing SA: young, male children (infants and toddlers) and presence of any immune suppressive condition such as prematurity, low birth weight, small size for age and sickle cell haemoglobinopathy.[7] Reveille reports that the incidence of SA in people living with HIV is similar to the incidence of SA in the general population, but that SA in the former group is usually caused by atypical organisms.[8]

Pathophysiology

Septic arthritis occurs from haematogenous spread, direct inoculation, spread from nearby osteomyelitis or surrounding soft tissue infections.[3] In the case of haematogenous spread, bacterial seeding occurs with subsequent lodging in highly vascular joint synovium.[9]
In neonates and children less than 18 months of age, transphyseal blood vessels allow communication between the growth plate and epiphyseal cartilage supplying a route for bacteria to spread from an osteomyelitic focus in the metaphysis to the epiphysis and subsequently to the joint lumen and vice versa.[9,10] Direct inoculation can occur through animal bites, penetrating injuries or medical procedures such intra articular injection of medicine.

Irreversible damage to the joint articular cartilage is caused by bacterial toxins, proteases from synovial cells and the increased pressure from pus formation within the joint capsule.[3,6,11] This increased intracapsular pressure can lead to avascular necrosis and is especially seen in the femoral head if SA is not promptly treated. Joint destruction happens within 8 hours after bacterial inoculation, emphasising the need for urgent diagnosis and management of SA.

Clinical features

Clinical features of SA are non-specific. Children can present with a warm, tender, red and swollen joint which fits the clinical manifestations of a number of other joint pathologies.[2] As the infection progresses, symptoms can rapidly progress over a few hours. Joint inflammation causes stretching of the joint capsule leading to pain with a limited range of movement in the affected joint as well as fever and malaise. SA of the lower limb can present with pseudo paralysis and refusal to weight bear. In neonates, signs and symptoms of SA can be absent. Neonatal patients can present with irritability, malaise and refusal to feed. Clinicians should thus keep a high index of suspicion in this age group.[5] In contrast to older children, neonatal patients with SA can have more than one joint involved.[5]

Differential diagnosis

Transient synovitis (coxitis fugax): this condition presents in a child between the ages of 3 to 8 years of age. It is a self-limiting condition that is managed without antibiotics and non-operatively. Patients present with acute onset of hip pain, refusal to weight bear in the absence of fever. Kocher criteria can help to differentiate this condition from SA. Transient synovitis is plausible when no predictors are found.[2]

Juvenile idiopathic arthritis: this condition is typically a polyarticular arthritis and has a gradual onset of symptoms. Joints are warm and swollen but not especially painful and the arthritis is symmetrical.[2]

Other differential diagnoses include slipped capital femoral epiphysis, malignancy, osteomyelitis, pyomyositis and cellulitis.

Causative Organisms

Staphylococcus aureus remains the most common cause of SA. In recent years, Panton-Valentine leucocidin (PVL) has been identified as a cytotoxin produced by some strains of *Staphylococcus aureus*. PVL induces pore formation in leukocyte cell membranes thus acting as a virulence factor. PVL producing *Staphylococcus aureus* SA are associated with longer hospital admissions, complicated infections with higher rates of septic shock, prolonged antibiotic use and a greater number of surgical interventions.[3,12])

Other common pathogens include *Kingella kingae*, *Streptococcus pneumoniae, Streptococcus pyogenes and Haemophilus influenzae* type B, Table 1.[2–4,13]

Atypical organisms that cause SA in immunosuppressed children in South Africa include Group B streptococci, *Escherichia Coli*, *Pseudomonas aeruginosa* and *Salmonella spp*.[2,4]

In cases of fastidious organisms, PCR can be performed on pus. This technology is not routinely available in routine laboratories.[2,5] In the South Africa context, pus should also be sent for tuberculosis diagnostic testing.

Table 1: Common bacterial pathogens associated with septic arthritis in children.[14]

Age group	Bacterial pathogen
Neonates	*Staphylococcus aureus* Group B streptococcus Gram-negative bacilli
Infants and toddlers (3 months to 3 years)	*Staphylococcus aureus* *Kingella kingae* Group A streptococcus *Streptococcus pneumoniae* *Hemophilus influenzae* (if not vaccinated)
Children (>3 years to 11 years)	*Staphylococcus aureus* *Kingella kingae*
Adolescents (>11 years to <18 years)	*Staphylococcus aureus* Gonococcal infections (in sexually active patients)

Laboratory studies

Initial studies should include a full blood count with differential, erythrocyte sedimentation rate (ESR), C reactive protein (CRP) and blood cultures (BC). These tests are helpful in making a diagnosis but alone cannot make a definitive diagnosis. Normal values of these tests do not exclude septic arthritis.

In 1999 the Kocher criteria were developed to aid in differentiating between SA and transient synovitis of the hip in children.
The criteria included the following four parameters:

a. history of fever,
b. non-weight bearing,
c. ESR of more than 40mm/h and
d. serum white blood cell count of more than 12 000 cells/mm^3.

According to the number of positive parameters present, patient probability of having SA of the hip is categorized into low or high probabilities.[2] A diagnosis of transient synovitis is plausible when no predictors are found.[2] In recent years, C reactive protein (CRP) levels of more than 20 mg/L have been added to the Kocher criteria as this laboratory test generally has a quicker turn-around time. The predicted probability of SA for the Kocher criteria ranges from 59 to 99.6% and remains similar when CRP is added, **Table 2**.[2]

Table 2: Number of Kocher criteria predictors present and predicted probability of septic arthritis.[15]

Number of predictors from Kocher criteria present	Predicted probability of Septic Arthritis (%)
0	<0.2
1	3.0
2	40.0
3	93.1
4	99.6

Imaging

Imaging of affected joint should commence with plain radiographs. This will aid in diagnosing osteomyelitis, fractures or neoplasms as the cause of a painful joint. Acute septic arthritis will most likely have normal radiographs aside from soft tissue swelling. In neglected SA, articular cartilage destruction will be evident by joint space narrowing and subchondral bone erosion.[3] It should be stressed that plain radiographs are not sufficiently sensitive to diagnose or exclude SA.[7]

Ultrasound is a rapid, non-invasive, non-irradiating test which is helpful in demonstrating joint effusions. It is especially helpful when assessing deep joints such as the shoulder and hip where palpation cannot reliably detect a joint effusion. An ultrasound where no joint effusion is detected within the first 24 hours after onset of symptoms, should be interpreted with caution. Ultrasound is unable to differentiate between SA and transient synovitis.[3,7]

Magnetic Resonance Imaging (MRI) is the gold standard imaging method in SA. It has a high sensitivity and specificity to detect SA, especially in early phases of the disease, **Figure 1**. MRI is also indicated when SA of more than one joint is suspected as it will aid the surgeon in planning surgical intervention. Furthermore, avascular necrosis may complicate SA of the hip and MRI can delineate early ischaemia. In SA of the shoulder and elbow there is a high risk of osteomyelitis and MRI can diagnose these complications.[3,7]

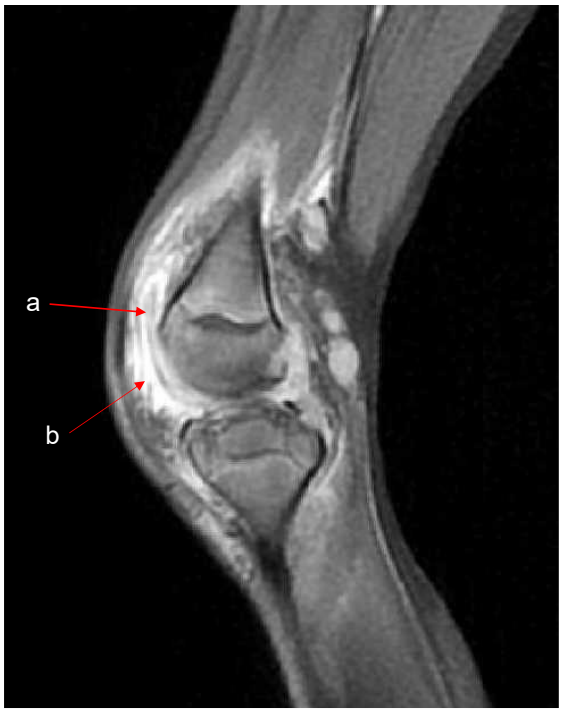

Figure 1: This image is an MRI scan of the right knee from a 6-year-old boy with septic arthritis. It shows (a) joint effusion with (b) extensive thickening and enhancement of the synovium.

Management

The mainstay of treatment involves prompt drainage and debridement of purulent material from the joint space and early treatment with antibiotics.[16]

Surgical management

Joint(s) affected by SA should be drained. There are three methods to achieve this: arthrocentesis, arthroscopy and arthrotomy. A systematic review done by Gigante et al. was unable to find consensus on the type of drainage procedure nor the timing of the intervention for SA for different joints. The authors did however find that surgical intervention becomes necessary when the clinical picture does not improve, and CRP does not decrease within 24 hours of antibiotic use.[7]

Medical management

In immune-competent hosts, empiric antibiotics that cover Gram-positive organisms should be selected based on local antibiogram patterns. Antibiotics should also cover *Staphylococcus aureus* SA until culture results become available. In immune-suppressed hosts, broad spectrum antibiotics which cover both Gram-positive and Gram-negative organisms should be selected based on local antibiogram patterns.

Evidence regarding the choice and duration of antibiotic treatment is sparse as no randomised control trials have been conducted thus far.[16] There is also no information available with regards to optimal duration of antibiotic use, timing of switching from intravenous to oral antibiotics and whether oral treatment is non inferior to intravenous treatment.

Donders et al. recommend that antibiotics should only be commenced once pus has been sampled for microbiology investigations unless a patient has signs of systemic sepsis, in which case antibiotics should be administered without delay.[2] The reason for this being that antibiotics administered before pus sampling decreases the culture yield and thus appropriate antibiotic therapy which prolongs a patient's admission to hospital. Gigante et al. does not differentiate between the timing of empiric antibiotics, but state that antibiotics should be initiated immediately.[7]

Empiric antibiotic therapy should be changed to definitive antibiotics once sensitivity results become available. The duration of antibiotic therapy remains unsure, with authors agreeing that the patient response and normalisation of CRP should be the main indicator when selecting duration of therapy.

Conclusion

SA requires urgent recognition and treatment to avoid disability. The most common pathogen for SA in children remains *Staphylococcus aureus*. Treatment involves antibiotic therapy and surgical drainage.

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A REVIEW OF CYTOMEGALOVIRUS (CMV)

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Abstract

Human cytomegalovirus (CMV) is a ubiquitous virus that has a significant impact on the health of children in Africa and globally. It can be acquired antenatally in utero (cCMV) or postnatally (pCMV) via breastmilk, genital secretions, or blood products. CMV can also be transmitted via solid organ or hematopoietic stem cell transplants.

Clinical presentation of CMV varies based on the age and immune status, with children living with HIV exhibiting significant complications from co-infection.
Most children with cCMV are asymptomatic but may develop deafness and neurodevelopmental delay later in childhood. pCMV in preterm or low birth weight babies may lead to pneumonitis, necrotizing enterocolitis, or a severe sepsis-like syndrome.

Ganciclovir and valganciclovir, its oral prodrug, are the only anti CMV drugs available in most African countries though their use is not widespread. New drugs and vaccines are being developed to improve treatment options and prevent the transmission of CMV. Strategies to manage cCMV include the use of longer durations of ganciclovir as a treatment option to prevent deafness.

Introduction

Though Human Cytomegalovirus (CMV) infection is ubiquitous and is usually asymptomatic or presents with a mild mononucleosis like disease, congenital infection and infection in immune compromised children can cause complex and life-threatening disease. CMV infection may contribute to poor health outcomes in adults and children without immune deficiency.[1]

The aim of this article is to review the various clinical presentations of CMV in children and to highlight advances in the prevention, diagnosis and treatment of CMV as well as to describe future innovative strategies that are being explored.

Epidemiology

Human CMV, also known as human herpesvirus 5, is a member of the herpesvirus family (Herpesviridae), the beta-herpesvirus subfamily (Betaherpesvirinae), and the Cytomegalovirus genus. The viral genome contains double-stranded DNA that range in size from 196 000 to 240 000 base pairs encoding at least 166 proteins and is the largest of the human herpesvirus genomes. CMV virus establishes latency by persisting in leukocytes and tissue cells after a primary infection. It can be intermittently shed leading to symptomatic infection especially if the host becomes immune suppressed.[2]

CMV can be acquired antenatally in utero (cCMV) or postnatally (pCMV) via breastmilk, genital secretions, and blood products. CMV can also be transmitted with solid organ or hematopoietic stem cell transplantation. The incubation period for horizontally transmitted CMV infections is highly variable. Infection usually presents 3 to 12 weeks after blood transfusions and between 1 and 4 months after organ transplantation. For vertical transmission through breast milk in preterm infants, the median time to onset of CMV viruria is 7 weeks (range, 3–24 weeks).[3]

CMV infection results in various clinical presentations dependent on the age and immune status of the child.[4] Older infants can acquire CMV at day care centres through direct contact with virus containing secretions and transmit the virus to their mothers or day care staff.

Pathogenesis

CMV establishes latency in its host, but reactivation can occur in response to various stimuli, and this results in infection of new cells causing end organ infection. Reactivation causes the release of cytokines such as tumour necrosis factor alpha and interferon gamma resulting in inflammation. CD8+ T-cells play a critical role in controlling CMV infection and disease. Therefore, in patients with T cell deficiency viral replication is uncontrolled and results in excessive shedding of CMV.
Major disease from CMV is often linked to inflammation, including pro-inflammatory cytokine production and is usually limited to situations where the immune system is significantly suppressed or still immature.

Diagnosis

CMV can be diagnosed by detecting the virus (culture, molecular testing such as PCR or viral load) or by detecting antibodies to CMV. The diagnosis is complicated by the ubiquity of the virus, the high rate of asymptomatic shedding and the frequency of reactivation.

Most laboratories now use quantitative real-time polymerase chain reaction (PCR; viral load) tests to diagnose active CMV disease and monitor response to therapy. Testing can be done on a range of specimens including cerebrospinal fluid, amniotic fluid, human milk, aqueous and vitreous humor, urine, saliva, nasopharyngeal and tracheal aspirates as well as blood; however, detection of CMV DNA does not always indicate disease. The test is usually done on urine or saliva for the diagnosis of cCMV. Dried blood spots have been shown to have a low sensitivity for diagnosing cCMV.[5]

Viral load testing can be done using plasma or whole-blood specimens. Higher values are observed in whole-blood specimens compared with plasma. The same assay and specimen type should be used for monitoring patients over time.

COBAS AmpliPrep/COBAS TaqMan CMV test is a real-time PCR test that targets the polymerase gene and is calibrated to the World Health Organization (WHO) international standard to quantify the CMV load in plasma with a reported range from 137 to 9,100,000 international units (IU)/mL. Viral load is usually reported in IU/mL and as a logarithmic value. Viral load can be used to assess response to treatment.

CMV can be cultured using human fibroblast cells. Conventional culture may take one to six weeks to show cytopathic changes so has largely been replaced by shell vial culture which is faster. This technique involves low-speed centrifugation and detection of CMV early antigen prior to the development of characteristic cytopathic effects in tissue culture. The centrifugation of specimens increases the absorption of virus. Cell monolayers are then exposed to monoclonal antibodies against early antigen and antibody binding indicates "early" CMV replication within the cells.

Histologic examination of tissue is useful for the diagnosis of invasive disease. Diagnosis is based on the presence of inclusion bodies. These are usually basophilic intranuclear inclusions, although eosinophilic cytoplasmic inclusions have also been reported. The diagnosis of CMV in tissue sections can be confirmed with specific immunohistochemical stains.[6]

As CMV establishes latency and virus is shed periodically, the diagnosis of CMV related end organ disease relies on demonstrating the presence of CMV virus with clinical and or histological features that is suggestive of disease. Detection of viral DNA from the target organ can provide strong evidence that the disease is caused by CMV infection. Lung biopsies, while being invasive and usually only done post-mortem are the “gold standard” for diagnosing CMV pneumonia.[7,8]

Current and or prior infection with CMV can also be assessed through serology however CMV IgM may persist for several months and can be representative of past infection rather than current infection.

IgG avidity assays measure the binding strength between IgG antibodies and CMV. This can help differentiate a primary CMV infection from a past CMV infection. Following primary infection IgG antibodies have low binding strength (low avidity) but over 2 to 4 months they mature and then have high binding strength (high avidity). Avidity testing can be used in pregnancy.

Congenital CMV (cCMV)

CMV is usually transmitted antenatally from mother to child in the setting where the mother acquires CMV for the first time while pregnant or when CMV is reactivated during pregnancy. CMV IgM can be detected in pregnant women with non-primary infections, so it is often interpreted along with CMV IgG avidity. A positive IgM with a low IgG avidity implies a recent infection whereas a high IgG avidity implies a lower risk of infection to the fetus. cCMV is defined as detection of the CMV virus in urine or saliva of the neonate before 3 weeks of age. Previously, the earliest age that prenatal CMV infection could be detected in the urine by culture was 3 weeks thus providing the time to differentiate between perinatal and congenital infection. However, specimens now need to be taken as soon as possible after birth to confirm cCMV. Most neonates with cCMV are asymptomatic with around 10% being symptomatic.

Symptomatic neonates can present with jaundice, petechiae, purpura, hepatosplenomegaly, microcephaly, intracerebral (typically periventricular) calcifications and retinitis. Developmental delay and sensorineural hearing loss can occur among affected infants in later infancy and early childhood.

Both symptomatic and asymptomatic infants may go on to develop sensorineural hearing loss with around a third of symptomatic and 10% of asymptomatic neonates developing hearing loss. The majority of these neonates will have normal hearing at birth. [9] cCMV is the most common non-genetic cause of sensorineural hearing loss in resource rich countries. [10] Symptomatic neonates treated with 6 weeks of intravenous ganciclovir have shown improved hearing outcomes.[11] While treatment with valganciclovir for 6 months moderately improved hearing and developmental long term outcomes, there was no difference between 6 weeks versus 6 months of treatment on hearing outcomes at the 6-month time point.[12]

Recently analyses of initial samples of cCMV infants have identified a 16-gene signature associated with the development of sensorineural hearing loss with 92% accuracy[13] which could potentially lead to a biomarker predicting hearing loss.

Table 1: Prevalence of cCMV in various African countries

Author & Year of publication	Country / Region	Screening method	Special population	Prevalence
Schopfer 1978	Cote d’Ivoire	Urine culture	NA	28/2032 (1.4%)
van der Sande 2007	Gambia	Urine PCR	NA	40/741 (5.4%)
Mwaanza 2014	Zambia	Urine & saliva PCR	HIV	15/395 (3.8%)
Manicklal 2013	South Africa	Saliva swab PCR	HIV	22/748 (2.9%)
Otieno 2019	Kenya	Saliva swabs and DBS PCR	NA	39/1078 (3.6%)
Olusanya 2015	Nigeria	Saliva PCR	NA	10/263 (3.8%)
Kaye	Gambia	Urine PCR	NA	11/281 (3.9%)
Morgan 2003	Egypt	Urine PCR	NA	10/175 (5.7%)
Salwa 2011	Egypt	Urine culture	NA	2/178 (1.3%)
Pathirana 2019	South Africa	Saliva swab PCR	HIV + and HIV-	67/2685 (2.5%)
Tshabala 2018	South Africa	Saliva swab PCR	HIV+ and HIV-	18/302 (6%)

The prevalence of cCMV in Africa varies between 1.3-6.3%.[14] Table 1 shows the prevalence of cCMV in various African countries. This is higher than the global prevalence that is reported at around 1%.[15] Children infected with human immunodeficiency virus (HIV) are at higher risk of cCMV than those that are HIV-exposed but uninfected.[16] In addition, CMV may increase the risk of in-utero HIV transmission.[17]

Postnatal CMV (pCMV)

pCMV is defined as detection of the virus after 21 days of life with the exclusion of cCMV.[18] Healthy term neonates are usually asymptomatic probably as a result of protection induced by maternal antibodies.

Preterm (less than 32 weeks gestational age) and very low birth weight (less than 1.5kg)(VLBW) neonates are at risk of a severe sepsis like syndrome, pneumonitis, bronchopulmonary dysplasia and necrotizing enterocolitis (NEC) after acquiring CMV postnatally via breastmilk ingestion. [19] Almost no CMV is detected in colostrum but CMV DNA is increasingly detected in breastmilk from about three weeks and reaches a maximum limit at 4 to 8 weeks.[20] In a prospective cohort study that followed infants born to CMV positive mothers who were CMV negative at birth and followed for 14 weeks, about half the VLBW preterm infants became CMV infected and a fifth developed clinically significant symptoms. None of the infants included in this study received treatment for pCMV infection with valganciclovir or ganciclovir during the study period[21] Those that became CMV infected had longer hospital stays and more episodes of prolonged neutropenia. However, NEC and bronchopulmonary dysplasia were not increased in those that became CMV-infected.

An infectious mononucleosis-like syndrome with prolonged fever and mild hepatitis may occur in about 10% of immunocompetent older children and adolescents.

CMV and HIV

As previously stated cCMV is more common in children infected with HIV and can increase the risk of in utero transmission of HIV. In addition, it may impact on HIV-exposed and uninfected (HEU) children as highlighted by a study of Zambian HEU infants in whom cytomegalovirus infection was associated with poor growth and lower cognitive development. [22] In addition, children not on antiretroviral therapy (ART) can present with pneumonia, oesphagitis, colitis, retinitis, meningoencephalitis, or transverse myelitis.[23,24] A syndrome that includes fever, thrombocytopenia, leucopenia and mild hepatitis may also by typical of CMV infection.

Human cytomegalovirus is a plausible driver of immune activation and inflammation in immunocompromised people.[25,26] Higher levels of CMV-specific IgG are associated with atherosclerosis in adults living with HIV on ART.[27] In an Italian cohort of adults living with HIV virally suppressed on ART, CMV seropositivity was associated with a 2.3 -fold higher incidence of cardiovascular events.[28] In addition, in asymptomatic adults living with HIV that were treated with valganciclovir, inflammatory biomarkers that strongly predict myocardial infarction and arterial inflammation (sCD163 and sICAM-1) were decreased. [29,30] There is limited evidence on how CMV infection affects future atherosclerosis or cardiovascular outcomes in perinatally HIV-infected children.

CMV and TB

Recently the association between CMV and tuberculosis (TB) has been highlighted in a study that showed that infants who acquired CMV before 24 months of age had an increased risk of tuberculosis disease between ages 1 and 9 years with an adjusted hazard ratio of 4.2 (95% CI 2·0–8·8; p<0·0001). Those with a high cytomegalovirus viral load seem to be at the highest risk.[31] Interestingly, CMV was not associated with higher rates of tuberculin skin test conversion but rather progression from infection to disease. Another case control study in South African infants found that the presence of IFN-γ responses specific to cytomegalovirus were associated with activated CD8+ T cells and a 22-times increase in the risk of tuberculosis disease.[32]

CMV in children with Primary Immune deficiencies

Infants with a primary immune disorder of cellular function for example severe combined immune deficiency; natural killer (NK) cell disorders may also present with severe or fatal pCMV infection.[33]

CMV infection in children with NK cell disorders can lead to haemophagocytic lymphohistiocytosis (HLH). This is a life-threatening condition characterized by excessive immune activation and can be diagnosed clinically when patients meet five out of eight criteria: fever, splenomegaly, cytopenias affecting two or more blood lineages, hypertriglyceridemia and/or hypofibrinogenemia, haemophagocytosis, low/absent NK cell activity, hyperferritinemia and high soluble interleukin-2 receptor levels.[34] In addition, active and latent CMV infection induces sustained systemic inflammatory responses and immune dysregulation and predisposes patients to the development of autoimmune phenomena.[35] Persistent CMV can also drive progression to lymphoid malignancy. CMV retinitis, colitis and pneumonitis may also be seen in children with primary immune deficiencies.

Treatment

Antiviral therapy is not usually indicated for CMV infections in immunocompetent children. For symptomatic cCMV, treatment with ganciclovir or valganciclovir (depending on severity of illness) is recommended for 6 months and should be started within the first month of life and as soon as the diagnosis is confirmed. pCMV colitis and pneumonitis are also indications for ganciclovir treatment. Treatment is usually continued until there is clinical resolution but for at least 3 weeks and often up to 6 weeks.

Table 2 summarizes drugs that have been used to treat CMV. Only 5 drugs have been approved by the Food and Drug Administration for the treatment of CMV: Foscarnet (1991), Ganciclovir (1994), Valganciclor (2001), Cidofovir (1996) and Letermovir (2017).

Table 2. Drugs for the treatment of CMV infection

Drug	Route of administration	Toxicity
Ganciclovir	Intravenous	myelotoxicity + nephrotoxicity
Valganciclovir	oral	myelotoxicity + nephrotoxicity
Foscarnet	Intravenous	myelotoxicity + nephrotoxicity
Cidofovir	Intravenous	myelotoxicity + nephrotoxicity
Brincidofovir	oral	myelotoxicity + nephrotoxicity
Letermovir	Oral and Intravenous	none
Maribavir	oral	dyseugia, nausea, diarrhea, vomiting

Ganciclovir/valganciclovir (prodrug of Ganciclovir) phosphorylates CMV specific kinases and is incorporated into viral DNA and inhibits the viral polymerase by acting as a chain terminator. Resistance develops through mutations in UL97 that encodes for viral kinases or mutations in UL54 that encodes the viral polymerase.

Foscarnet inhibits the pyrophosphate binding site on viral polymerase while Cidofovir or Brincidofovir (its oral derivative) is also phosphorylated by cell kinases and acts as a chain terminator. Viral polymerase is the common target and certain mutations in UL54 can confer cross resistance to ganciclovir and Foscarnet. As a result of cross resistance as well as hematological and nephrotoxicity of currently available anti-CMV drugs, new drugs are being developed.

Letermovir inhibits the terminal phase of the viral lifecycle, targeting the sub-unit pUL56 of the terminase enzyme complex. It is CMV specific and does not have activity against herpes simplex virus (HSV). It can be given orally or intravenously and is currently approved for adult stem cell transplant recipients. Unfortunately, it has a low barrier for resistance and a single mutation can lead to resistance.[36]

Maribavir, a benzimidazole, is a competitive inhibitor of ATP binding to pUL97 (a protein kinase that phosphorylates itself and other proteins essential for the viral lifecycle).[37] Mutations in UL97 and UL27 confer resistance. pUL97 is needed for phosphorylation and antiviral activity of ganciclovir, thus ganciclovir and maribavir are antagonistic. It has in vitro activity against Epstein Barr virus (EBV) but not HSV 1 and 2, Varicella Zoster virus, Human Herpes virus 6 and 8. It can cause dysuegisia.[36] The primary indication for Maribavir is for latent infection and refractory CMV, however recurrence has been documented. It is available for compassionate use for children in the United Kingdom and resistance has already been described in this population.[38]

Passive human immunoglobulin (HIG) has been used to prevent mother to child transmission of CMV and delay progression of CMV disease in solid organ transplant patients. A monoclonal antibody that would mimic this response would be of great benefit. Trials are ongoing for TRL345 and this appears to be the most promising candidate. [39]

Sirtuins are host-targeted antivirals (HTAs) that are directed against the host cell processes upon which viruses are dependent. Compared to directly acting antivirals, HTAs have the potential to reduce or eliminate viral resistance and demonstrate broad-spectrum activity. First-generation HTAs, such as interferons, broadly activate the host's innate and adaptive immune responses (e.g. hepatitis B and C) but are limited by toxicity.

Prevention

Due to the ubiquity of CMV infection the development of a safe and efficacious vaccine is of great interest. However there are three major obstacles to achieving this goal: lack of clarity as to the best population to target for vaccination (pregnant mothers or breastfeeding infants) ; virological obstacles such as the latency of CMV, reactivation of CMV disease during periods of immune suppression and, CMV's ability to evade immune response by using cell to cell spread; and laboratory-based obstacles such as unknown correlates of protection and a lack of animal models available for experimentation. [40,41]

The earliest published efforts were in the mid-1970s which used attenuated virus but these did not provide sufficient clinical protection. [42,43]While an increasing number of vaccine candidates has been developed none have, as yet been licensed. Live-attenuated vaccines based on an attenuated AD169 strain are currently undergoing a phase 2 trial in Japan. [44] Subunit vaccines that contain only the antigenic parts of CMV have been described since the 1990s.[45] However, these vaccines often need boosting or combination of an adjuvant due to their poor immunogenicity and the most recent trial from 2016 showed a vaccine that was safe but not efficacious.[46] Other methods that have been tried include virus vectored vaccine using modified Vaccinia Ankara or lymphocytic choriomeningitis virus; chimeric peptidic vaccines which place pathogenic antigen coding genes in safe organisms and more recently messenger RNA vaccines which use similar methodology to that used in the successful Covid vaccines .[47-49]

Valganciclovir have been used to prevent cCMV in CMV positive pregnant women.[50]
Hyperimmune globulin did not consistently prevent cCMV in pregnant women who were CMV positive.[51]

Conclusion

In summary, CMV is an ubiquitous virus that may often be asymptomatic but can cause a significant burden of morbidity in children through various clinical presentations. Diagnosis can be made on multiple specimens and usually requires molecular techniques as well as clinical or histological presence of disease. There are relatively few drugs licensed to treat CMV, however new drugs are being developed and vaccines to prevent CMV infection are in the pipeline.

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LABORATORY MATTERS

LABORATORY CHARACTERISTICS OF *STREPTOCOCCUS PNEUMONIAE*: A PERSISTANT PATHOGEN

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Abstract

Despite advances, such as the availability of an effective vaccine against *Streptococcus pneumoniae*, the organism still contributes significantly to morbidity and mortality worldwide, especially in children. It is crucial that accurate identification and susceptibility testing of the organism occurs, to allow for accurate estimation of disease burden, development of vaccines, and initiation of optimal treatment. Herein the laboratory diagnosis, susceptibility testing and treatment of *S. pneumoniae* is discussed.

Background

Despite the introduction of vaccines against *Streptococcus pneumoniae,* it still contributes significantly to morbidity and mortality worldwide.[1] It causes invasive disease including meningitis, bacteraemia, or infection of other sterile sites such as empyema and pericarditis.[2,3] Less severe, but still important non-invasive manifestations include pneumonia in the absence of bacteraemia, otitis media and sinusitis.[3]

It is estimated that in the year 2000, pneumococcal infections were responsible for 600 000 deaths in HIV-uninfected children, and an additional 95 200 deaths in HIV-infected children.[1] After 2010, many countries with a high burden of pneumococcal disease and who were eligible for vaccine funding from Gavi (the Global Vaccine Initiative Alliance), introduced the pneumococcal conjugate vaccine (PCV) as part of their national immunization programme.[1] Between 2000 and 2015, deaths due to pneumococcal infection declined by 51% in HIV-uninfected children, and 71% in HIV-infected children.[1]

However, despite this large-reported reduction in the proportion of disease from 2000-2015, in 2015, 294 000 HIV-uninfected and 23 300 HIV-infected children are still estimated to have died, highlighting the continued role pneumococcal disease still plays in childhood mortality.[1] India, Nigeria, The Democratic Republic of Congo, and Pakistan accounted for approximately 50% of these deaths in 2015, and sustained efforts to increase PCV vaccine coverage in these countries and others that have not yet introduced the vaccine, is necessary to combat the burden of disease particularly in children of low socio-economic status who are at increased risk of death.[1]

Misidentification of *S. pneumoniae* not only affects the estimation of disease burden, it also affects the development of vaccines against prevalent strains, and can also delay the initiation of optimal treatment. This is a frequently encountered problem in developing countries that still largely rely on culture-based methods for the identification of this organism. This article summarises the laboratory characteristics of *S. pneumoniae* and briefly expands on susceptibility testing and its implications in the treatment of this important pathogen, thereby providing a brief summary for clinicians and laboratory personnel alike.

Laboratory Diagnosis

Microscopy and culture are used commonly in diagnostic labs for identification. Gram stain typically reveals lancet-shaped, gram-positive diplococci (pairs) or cocci in short chains, Figure 1A. Most isolates contain an extracellular polysaccharide capsule which can also be visualized on Gram stain (Figure 1A) and by the Quellung reaction. The Quellung reaction is the gold standard for serotyping *S. pneumoniae*. The technique involves exposing the pneumococcus to anticapsular antisera resulting in visual enhancement of its capsule which appears as a halo surrounding the bacteria when viewed under a microscope.[3,4,5]

S. pneumoniae can be cultured on blood and chocolate agar plates that are incubated for 24-48 hours at 35-37° C. Growth is enhanced in 5% CO_2. Colonies appear small and grey with a surrounding green zone, caused by partial destruction of red blood cells, that is given the name α-haemolysis, Figure 1B.[3,4] These colonies often develop a central depression resulting in a draughtsman appearance, Figure 1B.[3,4] Some serotypes, for example serotype three, produce very mucoid colonies. *S. pneumoniae* is differentiated phenotypically from other α-haemolytic streptococci like viridans streptococci by optochin susceptibility.[3,4] Optochin (ethylhydrocupreine) is an antibacterial agent only used for laboratory identification of *S. pneumoniae.*[3,4] Unlike viridans streptococci, *S. pneumoniae* typically display optochin susceptibility (zone size ≥ 14 mm) (Figure 1C) although occasional optochin-resistant isolates have been reported.[3,4,6] Additionally, bile solubility testing, can be used when results of optochin testing is equivocal, and is based on accelerated autolysis of *S. pneumoniae* in the presence of sodium deoxycholate.[3,4]

Matrix-assisted laser desorption ionization time-of flight (MALDI-TOF MS) mass spectrometry is an alternative automated method for identification of *S. pneumoniae*. However, this method is limited in its ability to accurately distinguish between *S. pneumoniae* and the *S. mitis* group due to their genetic similarities. Distinction between these species has been improved by the combined use of a novel algorithm with an expanded MALDI Biotyper database.[7] Some laboratories will still confirm these results using optochin susceptibility. [8]

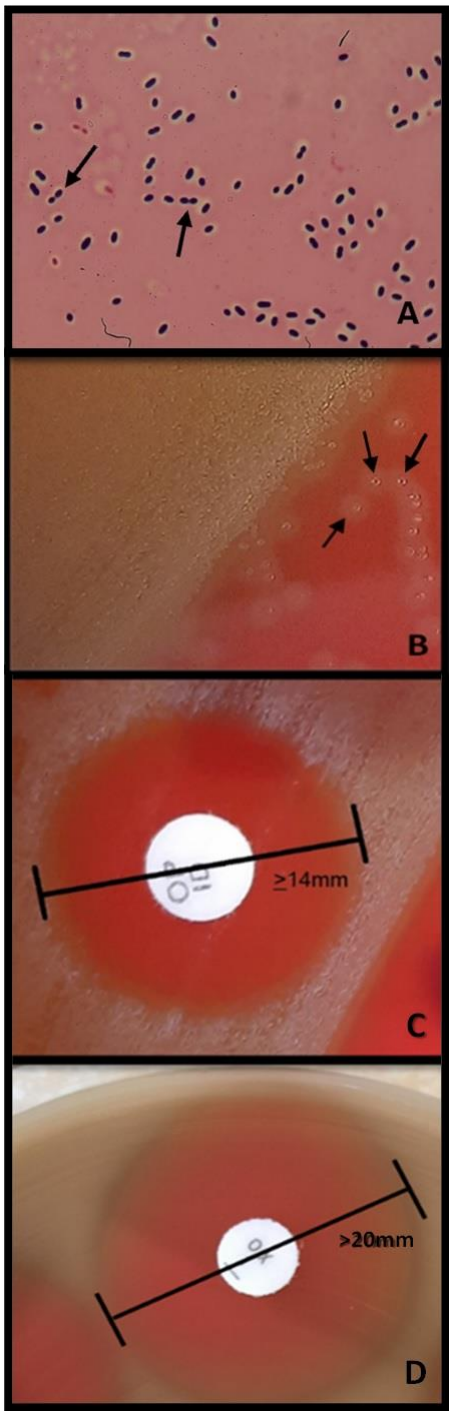

Figure 1: Routine tests performed in the diagnostic laboratory for identification and susceptibility of S. Pneumoniae
(A) *Gram stain showing lancet shaped gram-positive diplococci (purple) with surrounding capsule (arrow).*
(B) *S. pneumoniae colonies with draughtsman appearance and surrounding green zone of α-haemolysis on a blood agar plate*
(C) *Optochin susceptibility testing indicating that the isolate is S. pneumoniae due to a zone of inhibition ≥14 mm around the optochin disc*
(D) *Oxacillin disc diffusion screening test with an oxacillin inhibition zone >20mm indicating absence of β-lactam resistance mechanisms.*

Serotyping is an important tool for the surveillance of *S. pneumoniae*. This is necessary for monitoring the spread of disease and development of vaccines against the most prevalent disease-causing serotypes.[9] Geographical distribution, age of affected population and virulence is dependent on the specific serotype identified.[9] Ninety-eight different pneumococcal serotypes have been identified. based on the composition of the polysaccharide capsule.[10] Various methods for serotyping exist, these include capsular typing using antisera (Quellung reaction), MALDI-TOF MS and molecular techniques based on sequencing of the capsule gene.[10] Molecular methods include PCR, immunoblot, microarray and whole genome sequencing.[9,10]

Limitations of culture include the autolysing and fastidious nature of the organism. Additional factors such as low bacterial load of the organism within the sample, delays in transport, inoculation, and incubation of samples as well as the initiation of antibiotic therapy prior to sample collection can lead to false negative culture results. To counteract these limitations, non-culture-based diagnostics have been developed and include:

1) Immunochromatographic antigen detection tests (ICT). These are lateral flow assays and have a rapid turnaround time. They include the BinaxNOW assay which is a urinary antigen test that detects the C-polysaccharide cell wall antigen common to all *S. pneumoniae* serotypes.[4] They have also been used in non-urine samples such as cerebrospinal fluid and pleural fluid.[4] Their role is mainly as an adjunctive tool for diagnosis. Reports of cross-reactivity with other viridans streptococci have been described.[4] The value of the tests in diagnosing infections in children is questionable as nasopharyngeal carriage of *S. pneumoniae* can give rise to positive results.[4]

2) Molecular based methods including PCR. The common pneumococcal gene targets include *ply*, *lyt*A, *psa*A, and *pia*B. In culture negative cases a *lytA* PCR can be supplemented with *piaB* for better identification. However due to genetic similarity between *S. pneumoniae* and viridans streptococci, cross-reactivity has been described.[9] For this reason, other molecular methods including 16S rRNA sequencing, PCR followed by restriction fragment length polymorphism analysis (RFLP), Multi-Locus Sequence Typing (MLST) and whole genome sequencing are being investigated for accurate identification of *S. pneumoniae* but are beyond the scope of the routine diagnostic laboratory.[9]

Treatment and susceptibility testing

β-Lactam antibiotics such as penicillin, ampicillin, ceftriaxone, and cefotaxime, are the preferred treatment options for meningitis and serious non-meningitis infections. For meningitis, alternative agents include vancomycin. For non-meningitis infections, alternative agents include macrolides, tetracyclines and levofloxacin. The latter two, may be used for treatment in adults, however their use is contraindicated in children.[5]

Prior to 2008, penicillin minimum inhibitory concentration (MIC) breakpoints to determine susceptibility for all *S. pneumoniae* infections, were based on the more conservative values used for pneumococcal meningitis (0.06ug/ml).[11] As such, an increasing number of penicillin non-susceptible (non-wild type) *S. pneumoniae* isolates were reported.[11] These isolates showed reduced susceptibility to penicillin due to altered penicillin binding proteins, which results in lower affinity for β-lactams.[12] This

finding led to the increased use of broad-spectrum antibiotics in the treatment of these infections irrespective of the site of infection.[11] However, studies subsequently demonstrated that patients treated with optimal doses of parental penicillin for non-meningitis infections caused by these non-wild type strains with low level resistance (0.12-2ug/ml) had the same outcomes as patients treated with other antibiotics.[11] This led to the revision and differentiation of breakpoints based on the site of infection (meningitis versus non-meningitis), allowing clinicians to safely use parental penicillin to treat non-meningitis pneumococcal infection with low-level penicillin resistance (0.12-2ug/ml) again. For meningitis infections, the breakpoints for parental penicillin have remained low and unchanged at 0.06ug/ml, due to increased mortality when treated with penicillin in the setting of low-level resistance (MIC 0.12-2ug/ml).[11] However, in non-meningitis infections, the breakpoints for parental penicillin were revised to ≤2ug/ml for susceptibility. Similarly different breakpoints for susceptibility to cefotaxime and ceftriaxone exist based on whether the infection is a meningitis or non-meningitis infection.[12,13]

To exclude β-lactam resistance mechanisms, a screening test using disk diffusion with an oxacillin (1ug) disk can be performed. When the screen is negative (oxacillin inhibition zone ≥20 mm), all β-lactam agents for which clinical breakpoints are available, can be reported as susceptible, Figure 1D. When the screen is positive (oxacillin zone <20 mm), MICs to these β-lactams need to be performed to determine susceptibility.[12,13] When the oxacillin zone diameter is <8 mm, this could indicate resistance to broad spectrum antibiotics such as ceftriaxone/cefotaxime.[12] In these cases, an alternative, non β-lactam antibiotic should be added for serious infection until the MICs of the β-lactams are available.[5,12] An example of this would be adding vancomycin to β-lactam antibiotic therapy for pneumococcal meningitis until β-lactam susceptibility is confirmed.

Conclusion

Despite advances such as the availability of an effective vaccine against *S. pneumoniae* serotypes causing infection, the organism remains a formidable foe. It is crucial that accurate identification and susceptibility testing of the organism occurs to allow for accurate estimation of disease burden, development of vaccines against prevalent strains, and initiation of optimal treatment.

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THE LABORATORY DIAGNOSIS OF TYPHOID FEVER

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Abstract

Typhoid fever is a serious bacterial infection. Given its non-specific clinical presentation the laboratory diagnosis is critical. This article discusses existing and potential diagnostic methods. Culture particularly of blood remains very important but needs to be optimised in terms of volume and timing. Existing serological tests including the outdated Widal test and other serological tests generally lack sufficient sensitivity and specificity, though newer serological tests detecting immunoglobulin A to lipopolysaccharide, haemolysin E and other novel antigens, hold potential. Multiplex polymerase chain reaction on blood following a short pre-enrichment step also appears to offer excellent sensitivity and specificity but may be more suitable for research studies at this stage. The limitations of blood culture as the existing reference method are noted, and alternatives discussed.

Introduction

Typhoid fever is a serious bacterial infection affecting in 2010 an estimated 11.9 million people globally and causing an estimated 128 775 death.[1] The disease occurs mainly in

low- and lower middle-income countries (LMIC) in Asia and Africa and although the majority (> 70%) of cases occur in Asia, the incidence of disease may be highest in Africa with estimated rates of up to 537-557/100 000 population in East and Middle Africa.[1]

Typhoid fever is an epidemic-prone disease. Review of global data from 1990 -2018 shows that the number and size of typhoid fever outbreaks are increasing over time particularly in LMIC. The average size of outbreaks is much larger in Africa compared to other regions, suggesting late detection.[2] There have been several outbreaks in Africa since 2010, predominantly in southern and central Africa, largely due to a specific multi-drug resistant (MDR) strain or haplotype called H58 which appears to have emerged from Asia and spread globally.[3]

Typhoid and paratyphoid fever are caused by infection with *Salmonella enterica* serotype *Typhi* and *Salmonella enterica* serotypes *Paratyphi A, B* or *C* respectively.[4] These 'typhoidal' and 'paratyphoidal' serotypes infect only humans. In contrast the 'non-typhoidal' Salmonella serotypes tend to be associated with a variety of animal hosts and in humans typically cause gastro-enteritis though invasive disease can occur in the immunocompromised.[4] Paratyphoid fever is similar clinically to typhoid fever. It is thought to be less severe, though this has been challenged.[4,5] At present it is much less common than typhoid fever and seems to be rare outside of Asia where *S. paratyphi A* accounts for up to 20% of cases. The term enteric fever encompasses the clinical syndromes of both typhoid and paratyphoid fever though the term typhoid fever is sometimes used interchangeably, with the understanding that *S. paratyphi* serotypes may actually be the causative organisms.

The clinical features of enteric fever are non-specific.[6] In essence enteric fever presents as a prolonged undifferentiated febrile illness. Fever is a consistent finding in patients of all ages, though chills and rigors are much less common in children than in adults.[6] The classical early clinical signs of typhoid fever such as relative bradycardia and rose spots may be absent.[6] Other common non-specific early symptoms include headache, nausea, vomiting, constipation, diarrhoea, cough and abdominal pain.[6] Hepatomegaly and splenomegaly also occur frequently.[6]

Gastro-intestinal complications such as bleeding, granulomas/abdominal masses or perforation may occur in the 2nd or 3rd week, more frequently in older children or adults compared to young children, since these reflect a robust immune response to well primed Peyer's patches.[7] Transient pancytopenia is a classical feature of typhoid fever. While this can occur in children, leucocytosis also occurs, particularly among young children.[7] Other features more commonly found in young children < 5 years of age include anaemia, hepatitis and seizures.[6,7] Apart from variation with age, there may also be geographic variation in nature of some presenting signs and symptoms, e.g. relative bradycardia is reported in 50% of cases in sub-Saharan Africa compared to less than 20% of cases elsewhere.[6]

The differential diagnosis of typhoid fever can be extensive including bacterial sepsis, malaria, acute abdomen and other locally relevant febrile illnesses, such as dengue, brucellosis or rickettsial infections.[6]

The laboratory diagnosis is therefore critical to the accurate diagnosis and management of the individual patient as well as for an improved understanding of the epidemiology of the disease, and for limiting unnecessary antibiotic use as part of antimicrobial stewardship. This article will discuss the different options for laboratory diagnosis and suggest future directions and key actions.

Culture

Culture of *S. typhi* or *S. paratyphi* from sterile sites provides a definitive diagnosis of typhoid fever.[4] In addition, culture permits antimicrobial susceptibility testing and molecular typing that guides treatment and informs public health and infection control activities.

Blood is the preferred specimen type though stool or urine samples may yield positive results in about 30% of patients in the second week of illness.[4] Theoretically bone marrow culture is still considered the gold standard as the sensitivity is higher, up to 90% and less affected by prior antimicrobial therapy, but bone marrow is not a practical sample to collect in virtually any clinical setting. The sensitivity of blood culture for the diagnosis of typhoid fever is reported to be about 60%.[8] This modest sensitivity may be related to the low bacterial density encountered in typhoid fever where median *S. typhi* counts in blood may be only 1 ml (CFU/ml).[9]

Other factors apart from prior antimicrobial therapy that influence blood culture yield include duration of illness, as sensitivity is greatest in first week of illness, as well as the volume of blood sampled. Many studies either fail to document the volumes of blood cultured or use insufficient volumes. A recent systematic review and meta-analysis suggested that sensitivity increases by 3% for every 1 ml increase in blood volume between 1ml – 10ml.[8] A minimum volume of 7ml has been suggested as it should give a 90% chance of detection if median bacterial load is 0.3 CFU/ml, although ≥ 10ml may be necessary.[10]

The optimal volume for blood culture in general in children has been labelled a conundrum.[11] A number of age or weight-based recommendations exist but these differ considerably from each other e.g. for a child of 8 kg recommended volumes vary between 1.5ml and 10 ml.[11] In general, given the low bacterial density of typhoid fever, using volumes at the higher end of the recommended ranges would be preferred. Outside of the neonatal period (an age at which typhoid fever is rarely a concern) these volumes are all less than 1% of total blood volume, and so should be safely tolerated in most children.[11] In practice smaller volumes are frequently used, e.g. a recent community based prospective paediatric cohort study met their protocol specified requirements to collect at least 3ml in children ≤ 3 years and at least 5 ml in older children from 3 - 15 years of age and yet showed that a larger blood volume inoculated for culture was independently associated with blood culture positivity with an Odds Ratio (OR) of 2.82 (95% CI, 1.71–4.66).[12]

The particular laboratory methods used for culture of different specimen types may also play a role in successfully isolating the organism, e.g. for blood culture the medium should be a rich nutrient broth containing a lysing agent while the absence of automated blood culture systems in LMIC laboratories may contribute to a low yield and prolonged time to positivity.[13] The yield from stool culture may be increased by testing of multiple samples and the use of an enrichment step such as selenite broth.[4]

The isolation and identification of causative organisms is complex and requires appropriate laboratory protocols such as the use of selective media for non-sterile sites and multi-step algorithms for identification that include biochemical methods and serotyping. Culture has a slower turnaround time compared to serology or molecular methods, while newer tools introduced into diagnostic laboratories to speed up identification such as mass spectroscopy (MALDI-TOF) do not differentiate enteric fever pathogens from other *Salmonella* species. Biosafety protocols are particularly

important to prevent laboratory acquired infections among staff working with concentrated cultures of live organisms.[14]

Molecular methods

Theoretically molecular methods such as polymerase chain reaction (PCR) should be extremely sensitive and specific, and a number of PCRs have been developed. Initially these targeted *S. typhi* genes encoding known antigens such as flagellar H[15] and Vi antigens[16] but subsequently have expanded to encompass a wider range of unique targets in *S. typhi* and *S. paratyphi.*[17,18] In practice the sensitivity, typically in comparison to the imperfect reference methods of culture or a mixture of culture and serology, has been disappointing at a maximum of approximately 90%. This is possibly due to the low bacterial load in blood where the median *S. typhi* count is 1 CFU/ml or lessc[9,10] compared to the lowest limit of detection of many PCR assays of 4 CFU/ml.[10] The need for a multiplex PCR that also simultaneously identifies and differentiates *Salmonella paratyphi A* and invasive non-typhoidal *Salmonella* species aggravates this problem as multiplex PCRs tend to perform worse than conventional single target ones.[17]

Methods to increase sensitivity for the detection of *S. typhi* have been described, such as nested PCR,[20] as well as methods to enrich bacterial DNA concentrations by removing human DNA[21] and or by including a short period of incubation to permit bacterial growth before DNA extraction.[22] Recent use of such a pre-amplification incubation step together with a multiplex PCR for the detection of *S. typhi* and *S. paratyphi* A in patients presenting with a febrile illness of at least 3 days without a clear focus of infection showed an overall sensitivity and specificity > 92% when compared to optimised blood culture using verified though generally low blood volumes in an automated blood culture system.[19] A similar approach in Malawi in young children presenting with undifferentiated fever to a tertiary hospital showed an increase in case detection of 62-94%.[23] Molecular methods can also be applied to gut tissue samples collected at surgery in cases with intestinal complications where prior antibiotics and presence of normal gut flora may hinder culture based detection.[24]

Additional drawbacks of molecular tests include complexity, cost, and lack of susceptibility results. Turnaround time may be reduced compared to culture, provided testing is done on demand rather than being processed in batches or referred to a distant central laboratory. A simpler more affordable molecular method involving pre-concentration followed by Loop-mediated isothermal amplification (LAMP) has been described[25] and similar developments may overcome some of these drawbacks in the future.

Serology

In settings where microbiological investigations and culture are not easily available the diagnosis of typhoid fever is often based on serology, notably the Widal test, first described in 1896.[26] The Widal test is an agglutination test that detects antibodies in sera to O (Lipopolysaccharide (LPS)) and H (flagellin) antigens of *S. typhi*.[27] It is a rapid, inexpensive, and simple test. Theoretically the test should be repeated after 7- 10 days to demonstrate a four-fold rise in titre of antibodies, but usually interpretation is based on a single test performed at the time of presentation.[28] There is considerable variability in the performance of the test and this lack of standardisation results in poor reproducibility.[26]

The Widal test lacks both sensitivity and specificity. False negatives may occur early in disease, false positives may reflect previous infection or vaccination, or cross reactivity with antibodies resulting from other infections, including non-typhoidal *Salmonella* or other bacteria such as *Brucella*.[27] In endemic areas varying cut-offs have been used to determine positivity, ideally based on contemporary baseline values in the local community. Cut-off titres in use typically range from 1: 40 – 1:200.[29] However, even with customised cut-offs, Widal test results should be interpreted with caution, e.g. in a study of hospitalised adults in Vietnam with a very high prevalence of typhoid fever of approximately 30% in a setting where non-typhoidal salmonella infections and vaccination were rare and using moderately high cut-off titres of > 1: 200 or > 1: 100 for O and H antigens respectively, the positive predictive value (PPV) and negative predictive value (NPV) were only 86% and 90% respectively.[30] In general the use of the Widal test for the diagnosis of typhoid fever is not recommended.

Several alternative serological tests have been developed over the years. A Cochrane review in 2016 of commercially available rapid diagnostic tests included 37 studies with more than 5000 participants mainly from highly endemic areas in Asia. Sixteen different RDTs were assessed although 3 assays and their variants (TUBEX, Typhidot and Test-it Typhoid) constituted the majority of studies. The review reported only moderate diagnostic accuracy with sensitivities ranging from 70- 85% and specificities from 80-90% with no evidence of a difference between the average performance of the 3 common tests.[31]

More striking was the overall heterogeneity of results, and the overall poor quality of studies, e.g. most studies selected patients on the basis of a clinical suspicion of typhoid fever although the criteria for such suspicion were usually not stated, and less than one third of studies recruited patients presenting simply with fever, the patient population in whom the diagnostic test would most likely be applied. In addition,16 of 37 studies used a case control study design which is likely to overestimate diagnostic accuracy. Similarly, only 3 of 37 studies used bone marrow culture as part of the reference method. The systematic review concluded that none of the tests included were sufficiently accurate to replace blood culture, and that more robust evaluations of alternative RDTs were required. Other systematic reviews have highlighted similar concerns about the quality and heterogeneity of studies of alternative diagnostic tests, noting that key details regarding the reference test used, such as the volume of the biological sample cultured and the receipt of prior antibiotics, are not recorded in most studies.[32,33]

More recently various new techniques have been used to identify novel targets for use in next generation serodiagnostic tests.[34-37] The targets identified include LPS, haemolysin E (HlyE), cytolethal distending toxin B (CdtB), membrane preparation (MP), flagellin and various others. Tests detecting IgA isotype antibodies also seem to be more accurate compared to those detecting IgG or IgM antibodies, possibly because IgA is more transiently produced compared to long lasting IgG but is more specific and less prone to cross reactions than IgM.[35,38]

Recently a rapid point of care (POC) immunochromatographic test was developed using an existing commercial Dual Path Platform (DPP) technology that includes separate paths for sample and for conjugate, together with a portable digital reader.[39] Based on detection of IgA to HlyE and to *S. typhi* LPS, an initial evaluation of its use in a small group of adults in Bangladesh reported a sensitivity of 90% and specificity of 96% (compared to febrile endemic controls). Further studies are required, but this POC test could be a major improvement because of the rapid turnaround time, ease of performance and no requirement for specialised equipment or highly trained laboratory staff.

Future directions

To guide researchers and developers, detailed specifications for an improved typhoid diagnostic test have already been suggested.[40] Based on a literature review, a Target Product Profile (TPP) including both the minimal and optimal characteristics for 36 parameters, was drawn up and subsequently refined using 2 rounds of a Delphi survey with key stakeholders and experts. Among the main requirements for the TPP were that the target population should include patients of all ages presenting with undifferentiated febrile illness at any point in the disease to the lowest level of the health care system. The test should cover both *S. typhi* and *S. paratyphi*, require <1 ml of blood, cost ideally only $1 and have a sensitivity of around 90% and specificity ≥ 95%. Ideally such a POC test also needs to be part of a diagnostic and treatment algorithm that caters for other locally relevant febrile infectious diseases.

Part of the difficulty in developing and evaluating alternative diagnostic tests for enteric fever is the imperfect reference standard provided by culture of blood, or even of bone marrow. Using an imperfect reference standard might reduce the specificity of a novel diagnostic test i.e. the so-called "false positive" might actually be a true positive.

Potential solutions to this problem include the use of a composite reference standard or the use of a Bayesian approach with latent class analysis. A composite reference standard (CRS) combines multiple tests with good specificity thereby increasing the overall sensitivity, e.g. positive culture from blood or bone marrow or urine, or positive culture and/or positive PCR. The advantages of a CRS compared to other alternative reference standards are that it can be more clearly defined and is more straightforward to interpret. A recent meta-analysis motivated for the development of a CRS for Typhoid fever using clear standardised definitions to facilitate good quality prospective cohort studies of new diagnostics.[32] Another meta-analysis using a Bayesian network procedure with latent class analysis grouped tests according to the underlying principle i.e. antibody detection, antigen detection, PCR, etc., and despite the similar problem of highly heterogeneous studies was able to draw some broad conclusions as well as establishing a method for evaluating the performance of combinations of tests.[33]

Other novel diagnostic approaches are also being explored for the diagnosis of typhoid fever including proteomics,[41] transcriptomics[42] and metabolomics[43] though none have yet been translated into tests for routine clinical use nor are they likely to meet the Target Product Profile requirements in the near future.

Conclusions

There is a need to improve the laboratory diagnosis of typhoid fever. To maximise benefit from existing test methods, it is important to:

1. Optimise the yield from blood cultures by ensuring the collection of adequate volumes of blood- at least 7ml in adults, and at least 3ml in children ≤ 3 years and at least 5 ml in older children from 3 - 15 years of age. Cultures should also be taken at the time of presentation before the administration of antibiotics.

2. Ensure that routine diagnostic microbiology laboratories are capable of the complex tasks required for the culture-based isolation, identification and susceptibility testing of typhoidal *Salmonella* species from blood and other specimens. This might mean ongoing support in terms of adequate numbers of appropriately trained staff, development of updated standard operating procedures and test algorithms and a reliable supply of the necessary media and reagents. Smaller laboratories need to be supported by larger reference or public health laboratories who also play an important part in the control of outbreaks of typhoid fever by providing timeous and accurate surveillance reports, including ideally molecular typing of strains.

3. In areas where the Widal test remains the only practical diagnostic test available currently, its performance and interpretation should be critically assessed, and results applied with caution. Health systems need to ensure that patients at different levels of care have access to diagnostic tests for typhoid fever.

Improved diagnostic tests are also urgently required.

1. In particular, there is a need for a POC test with improved performance characteristics for use particularly among outpatients. Ideally this POC test should meet the suggested requirements of the Target Product Profile. It is not clear yet whether the Dual Path Platform POC test targeting IgA to HlyE and to LPS meets these requirements. However, the identification of a variety of other novel antigens and host immune responses holds potential for development of alternative rapid tests.

2. Molecular tests that offer improved sensitivity whilst also targeting and differentiating *S. typhi* and *S. paratyphi* as well as non-typhoidal salmonella species are required. While such tests may not be practical as routine diagnostic tests, they may be useful in research settings like epidemiological studies in high disease burden settings and as secondary endpoints in vaccine efficacy studies. Simpler molecular methods using LAMP amplification hold potential for future use as tests that can be performed closer to patients in smaller laboratories.

3. Studies of future potential diagnostic tests should be of high quality including improved study design, optimisation of blood culture methodology and accommodation of an imperfect reference standard.

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CASE REPORTS & MEDICAL IMAGES

ODYSSEAN MALARIA IN THE WESTERN CAPE PROVICE OF SOUTH AFRICA

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Abstract

Due to the unexpected diagnosis of Odyssean malaria, cases are often missed, and the presentation is usually delayed resulting in severe complications. What makes these infections so intriguing is the lack of travel history from patients who acquire the infection while living in a non-endemic malaria area. We report the first case of Odyssean malaria in a young girl from Cape Town who presented multiple times to the health care services with a non-resolving fever amongst many other non-specific symptoms.

Keywords: Odyssean malaria, *Anopheles* mosquito, Western Cape, severe malaria

Background

Laboratory confirmation of malaria is quintessential for the diagnosis of Odyssean malaria. The name Odyssean malaria is derived from Greek mythology where the Greek king Odysseus survived the Trojan War and then spent a decade wandering in the Mediterranean Sea trying to return home. Malaria transmitted through the bite of a mosquito that has passively travelled thousands of kilometres (by plane, road or train) from a malaria endemic area to survive until its next meal in a non-malaria area has been called Odyssean malaria, likened to this mythical king's journey.[1] We report a case of this rare kind of malaria in a child from Lotus River, a suburb of Cape Town, in the Western Cape Province of South Africa.

Case presentation

On the 25th of December 2021, we saw the index presentation of what was later discovered to be Odyssean malaria on a well-grown 7-year-old girl who is HIV negative and fully immunized. She initially presented with fever of 38.4°C, lethargy, diarrhoea and three seizures, one focal in nature. A series of investigations were performed including analysis of urine, stool, blood and cerebrospinal fluid following a normal computed tomography of the brain. Despite a normal full blood count (FBC), her inflammatory markers where significantly raised with a C-reactive protein (CRP) of 247mg/L. Her remaining blood work was unremarkable. The patient was commenced on ceftriaxone, a broad-spectrum intravenous antibiotic, and her 48hr CRP level improved to 53mg/L. Even though a source of infection was not identified, she recovered well and was discharged after five days of intravenous antibiotic cover. Despite conflicting evidence regarding performing electroencephalograms (EEGs) on patients presenting with febrile seizures, an elective EEG was booked for the 18th of January 2022.[2]

Approximately two weeks later, on the 14th of January 2022, the patient presented to hospital again with a two-day history of fever (39.8°C), vomiting and diarrhoea, backpain, poor appetite and lethargy. She was diagnosed with acute gastroenteritis and discharged on zinc and paracetamol after receiving rehydration therapy. She re-presented to the emergency unit on Sunday the 16th of January with ongoing fever, vomiting and lethargy and was diagnosed with acute otitis media and discharged on oral amoxicillin. The following day, marking the 4th presentation to health services in a period of a month, she was referred to the medical emergency unit with persistent lethargy after her elective EEG showed abnormal moderate encephalopathy.

Upon arrival at the medical emergency unit, she had a Glasgow Coma Scale of 14, she was pyrexial (38.5°C) and strikingly pale. She was haemodynamically stable with a 1/6 ejection systolic murmur and a 2cm hepatomegaly. Once again, her CRP was elevated (254mg/L), but the FBC on this admission showed a significant bicytopaenia with a haemoglobin of 5.4g/dL and a thrombocytopaenia of 59 x 10^9/L. She also tested positive for COVID-19. On recognition of the abnormal FBC, the laboratory astutely performed a thick and thin film which was positive for *Plasmodium falciparum* with a high parasitaemia of 9.3%—an indicator of severe malaria. Other markers of severity were renal impairment (urea of 11.6 mmol/L), impaired level of consciousness and history of seizures on first presentation. A subsequent diagnosis of cerebral malaria was made. The patient fully recovered after treatment with intravenous artesunate. However, we were curious about how this young girl who was without a travel history and living in a non-malaria-endemic area acquired this infection. An environmental investigation by The National Institute for Communicable Diseases of South Africa could not find a source of infection but suspected a vector source mosquito that potentially hitch-hiked in the luggage of a neighbour who travelled from Mozambique in the last week of December 2021.[3]

Discussion

Billions of dollars are invested annually into malaria research and global malaria elimination strategies. As a result, malaria deaths have significantly reduced in the past two decades. The COVID-19 pandemic however hindered some of the progress when 2020 saw a 12% global increase in malaria deaths compared to 2019.[4] In many developing countries malaria remains a leading cause of morbidity and mortality.[5] In South Africa, only certain areas are seasonally endemic to malaria (Limpopo, Mpumalanga and Northern KwaZulu-Natal); the risk of transmission being higher between September and May.[6] Technological advances in tourism and transportation however puts traveling and non-travelling South Africans at perennial risk of acquiring imported malaria and the more unusual Odyssean malaria.

Between the period of 1996 and 2004, 46 cases of Odyssean malaria were reported in the non-endemic Gauteng Province. This was followed by an 8-cluster outbreak of 21 cases between 2007 and 2013, and a 2-cluster outbreak of 5 cases in January 2021.[1] It is not surprising that all the cases were detected in the province of Gauteng as it is the hub for travellers from Sub-Saharan endemic Africa. Gauteng also lies within proximity to endemic provinces which makes windborne malaria transmission not impossible — Anopheles mosquitoes may cover up to 300km of distance on high-altitude flights.[7] The case of Odyssean malaria in the Western Cape is intriguing and to the best of our knowledge has not previously been reported.

True thrombocytopaenia, one of the signs of severe malaria, should be confirmed by examining a blood smear to exclude ethylenediamine tetra-acetic acid induced platelet clumping.[8] On a smear, the malaria trophozoite can be seen which is how the laboratory identified the infection in our case. The symptoms and clinical presentation of malaria are non-specific which made this laboratory process critical in this diagnosis of Odyssean malaria and lifesaving for this patient. Malaria should be suspected in any patient presenting with a fever in a malaria-endemic area. In a non-endemic area, investigating all patients with a fever would be costly, therefore clinicians should be trained to conduct a parasitological test where there is a fever with no obvious cause, especially in association with thrombocytopaenia, even in the absence of travel.[9]

The clinical symptoms of both malaria and bacteraemia overlap, and concomitant bacteraemia has been frequently reported in association with severe malaria in endemic areas with a notable increased risk of mortality in children.[10] Third-generation cephalosporins such as ceftriaxone are recommended as an add-on therapy with appropriate antimalarials in cases of severe malaria to cover concomitant sepsis.[9] Our patient showed a good clinical response and remained symptom free for two weeks after receiving five days of ceftriaxone. This is an appropriate response when third generation cephalosporins are used to treat gram negative/ gram positive sepsis but an unlikely response for a severe malaria infection. It is therefore possible that the malaria infection occurred after the presentation and treatment of the initial sepsis.

The vector source in our case was thought to be linked to Mozambique, one of South Africa's neighbouring countries where there is malaria risk throughout the year. The time period of the identified traveller, which is noted to be the last week of December, corresponds with the incubation period of malaria (which is a minimum of seven days) and the patients second clinical presentation on the 14th of January. This may suggest the possibility of the malaria infection occurring after treatment of the initial sepsis. Another possibility which should be considered is a mosquito bite during one of many play dates at a nearby diverse community that is occupied by individuals and families from South Africa and Sub-Saharan African countries with endemic malaria, who could have unwittingly transported an infected Anopheles mosquito from an endemic area along an 'Odyssean journey' of more than thousands of kilometres.

For the past two years COVID-19 has impacted all spheres of life (social, economic, psychological) including putting a major strain on the South African Health Department. Its dominating presence has resulted in delayed treatment-seeking behaviour due to movement restrictions and reduced healthcare-worker malaria awareness.[11] It is important to note that COVID-19 infection in children is often an 'incidental finding' and may not be the cause of severe illness in critically unwell children but rather a red herring that can further delay the true identification of unusual presentations of fever.

COVID-19 has also disrupted and complicated malaria control programmes at all levels.[11] The World Health Organization encourages the world to refocus on global control and elimination strategies even during the COVID-19 pandemic. Specifically for South Africa, the aim is to eliminate malaria (eradication of local transmission) by 2023.[12]

Conclusion

Modern transportation has made travel easily accessible. In the same breath, it's that accessibility that facilitates spread of infectious diseases to unlikely places and to global proportions. This case of Odyssean malaria is a stark reminder that malaria has no borders. Clinicians should consider and test for malaria in all patients presenting with an unknown cause of a fever, even during the COVID-19 pandemic where co-infection is possible in order to avoid delays in diagnosis and progression to severe presentations of the disease as in our patient. This should be promptly followed by notification, which is equally important, to ensure timely investigation by the malaria control programme.

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PUBLICATION WATCH

INTERVENTIONS FOR LOWERING THE RISK OF DIARRHOEAL DISEASE IN CHILDREN

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Approximately 2.0 billion people do not have access to safely managed drinking water, sanitation services, nor handwashing facilities in their homes. Unsafe water, sanitation, and hygiene (WASH) causes more than 800,000 diarrhoeal deaths per annum, with approximately 300,000 of these deaths occurring in children less than 5 years of age.[1]

One of the abstracts published in the last edition of the AfSPID Bulletin and presented at the recent World Society for Pediatric Infectious Diseases conference described the effectiveness of point-of-use chlorination on diarrhoeal risk reduction in children less than five years in a rural setting in Ethiopia. This study documented a significant 36% reduction in diarrhoea risk in the intervention arm compared to the control arm, demonstrating the importance of primary prevention measures for reducing the burden of childhood diarrhoeal disease.[2]

The publication in focus, a systematic review and meta-analysis provide present-day effectiveness estimates of WASH interventions on the risk of childhood diarrhoeal disease in low- and middle-income countries.[3]

A total of 124 studies were included in this analysis, including 23 new studies that had not been used in previous systematic reviews.

Compared to untreated water from an unimproved source, water treated at point-of-use by filtration, solar treatment or chlorination reduced the risk of childhood diarrhoeal disease by 50%, 37% and 34% respectively.

The overall impact of 20 sanitation intervention studies was a 24% reduction in the risk of childhood diarrhoeal disease. While the provision of basic sanitation with sewer connection resulted in a 47% reduced risk for diarrhoeal disease, provision of basic sanitation without sewer connection only achieved a 21% reduction in diarrhoeal disease risk.

Handwashing interventions that promoted handwashing or improved access to handwashing facilities and materials (41 studies), showed an overall reduction in childhood diarrhoeal disease risk of 30%

In conclusion, this timely study provides updated estimates of the impact of these important interventions on the risk of childhood diarrhoeal disease and re-affirms the importance of WASH interventions for improving global child health.

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THREE TRIALS EVAULATING THE EFFICACY OF TYPHOID CONJUGATE VACCINE IN ENDEMIC SETTINGS

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Abstract

In this review, the outcome of recent typhoid conjugate vaccine (TCV) efficacy trials among children in three low-income countries is presented along with their implication for pediatric infectious diseases' practice in settings where typhoid fever is endemic.

Background

Enteric fever is predominantly diagnosed in children, notably in the age group between 5 and 15 years of age. It is mainly caused by *Salmonella enterica serovar typhi* (typhoid fever) and *Salmonella enterica serovars paratyphi* A, B and C (paratyphoid fever) and communities with poor sanitary practice and inadequate water supply are at high risk. Populous nations with predominantly young inhabitants and developing economies mean that African, south and south-east Asian countries carry the highest burden of typhoid fever globally. Worldwide, nine million infections and more than 100,000 deaths occur due to typhoid fever each year. African countries account for 13% and 17% of global infections and deaths respectively, each year.[1]

As with other infections, the treatment of typhoid fever is facing challenges from antimicrobial resistance, notably a rising rate of fluoroquinolone resistance. Of particular concern is the development of the R717Q mutation in the *acrB* gene, responsible for conferring resistance to azithromycin which is used to treat infection caused by extensively-drug resistant isolates.[2] Though clinical bacteriology laboratories are in short supply in African countries, studies note a similar trend of worsening resistance to ampicillin, trimethoprim-sulfamethoxazole and chloramphenicol and fluoroquinolones.[3]

In these settings immunisation has a significant role to play in typhoid prevention. Two previously evaluated vaccines proved to be moderately effective against typhoid fever. The oral attenuated Ty21a vaccine administered in four doses with booster every 5 years has an efficacy of 48% while Typhim ViCPS, a polysaccharide vaccine that is administered with a solitary dose and boosted every 2 years, has an efficacy of 55%.[4,5] Since 2017 the Typhoid Vaccine Acceleration Consortium has conducted three large, TCV efficacy trials in Nepal, Bangladesh and Malawi.[1] This conjugate vaccine has the advantages of single dose administration and because it is not a live vaccine can be considered for use in immune-compromised children.

Lessons from the three recently conducted conjugate vaccine trials

A comparison of the baseline country and sociodemographic characteristics of the study populations of the three trials is summarized in table 1.[1,6,7]

Table 1: Comparison of the populations in which the three trials were located and characteristics of the study populations

Parameter	Nepal	Bangladesh	Malawi
Incidence of typhoid fever per 100,000 person-years	484 - 615	200	184
Under five mortality rate (per 1000 live births) 2020	30.4	29	38.6
Age range for study population	9 months – 16 years	9 months – 16 years	9 months – 12 years
Control vaccine used in the control group	Meningococcal A conjugate vaccine	Japanese encephalitis vaccine	Meningococcal A conjugate vaccine

The primary outcome in the three trials was blood culture-confirmed typhoid fever occurring at any time during the study period (Malawi and with secondary outcomes being immediate and long-term adverse events, immunogenicity with anti-Vi IgG titres (Nepal and Bangladesh trials), indirect vaccine protection of non-vaccinees and total protection by Vi-TT against paratyphoid fever (Bangladesh trial). While investigators in the Malawi trial carried out both intention-to-treat (ITT) and per-protocol (PP) analyses, the Nepalese and Bangladeshi research teams only performed per-protocol analyses. The trials were adequately powered, including large numbers of children aged 9 months and older (Table 1).

The efficacy of TCV in the Nepal and Bangladesh trials was 81.6% and 85% respectively. The decision to include an ITT analysis in the Malawi trial resulted in different efficacy outcomes, 80.7% in the ITT analysis and 83.7% in the PP analysis. The lower efficacy in the ITT analysis was due to the inclusion of break-through typhoid fever occurring before day 14 post-vaccination when vaccine-induced protection was still sub-optimal. Lower efficacy was noted in younger children in all three trials: 65% in the 2-5-year-old group vs 87.5% in the 5 -16 years age group in Nepal, 80% in both the under 2 years and the 2–4-year age group compared to 88% in the 5–16-year age group in Bangladesh, and 74% in children younger than 5 years vs 84–88% among children aged 5–12 years in Malawi.

All three trials demonstrated the safety of the TCV with no vaccine-related adverse events at post-vaccine day 7. Investigators in the Malawi trial included a more detailed safety assessment with surveillance of adverse events assessed at 30 minutes, 7 days, 28 days and 6 months but with similar conclusions.

Of concern was the detection of four isolates resistant for ciprofloxacin among the 74 culture-proven episodes of typhoid fever in the Malawian study population. All four isolates were multi-drug resistant (MDR) or resistant for amoxicillin, trimethoprim-sulfamethoxazole and chloramphenicol. While none of the 41 isolates in the Nepal trial were MDR, most strains were resistant to ciprofloxacin and two had reduced susceptibility to azithromycin. In Bangladesh, investigators identified 475 blood culture-proven episodes of typhoid fever, one in four being MDR and 10% being resistant for ciprofloxacin. The confirmation of fluoroquinolone- and azithromycin non-susceptible strains in such young populations should raise the alarm for stepping up antimicrobial stewardship efforts in these countries.

In the sub-group analysis of the children in the Bangladesh and Nepal trials, high levels of immunogenicity were demonstrated with 99% seroconversion rates on day 28 post-vaccination. The research team in Dhaka, Bangladesh did not observe significant indirect vaccine protection among non-vaccinees.

The efficacy of TCV among malnourished and HIV-infected children was not determined in these three trials. As shown in Table 2, rates of malnutrition and HIV infection, two immune-compromising conditions which can affect immune response and vaccine efficacy among vaccine recipients, are high in the three countries that hosted these trials. Failure to include these vulnerable patient groups results in an incomplete understanding of vaccine efficacy. However, investigators in Malawi are currently conducting a TCV efficacy study among children living with HIV infection. This ongoing study will evaluate the administration of one- or two-doses of the TCV when co-administered during routine 9-month and 15-month vaccination visits.

Table 2: Country statistics of child health in the three study countries

Parameter	Nepal	Bangladesh	Malawi
Under five mortality rate per 1000 live births	28	29	39
Prevalence of stunting	30%	30%	37%
Country prevalence of HIV infection, 2020	0.1%	0.1%	8.1%

The outcomes of these three trials heralds the start of improved prevention against typhoid fever in low- and middle-income countries. The high incidence rates of typhoid fever, with most endemic sub-Saharan African countries recording rates of 50 and more cases per 100,000 population, and the favourable safety and efficacy profile of TCV justifies its inclusion in routine vaccination programmes in typhoid endemic countries.[8]

Monitoring the impact of TCV in endemic countries after its routine introduction using annual hospitalization and mortality rates as well as disability-adjusted life years (DALYs) will further strengthen prevention efforts. The World Health Organization currently recommends the use of a single dose of TCV for ages 6 months–45 years in endemic countries with further catch-up campaigns for children aged 15 years or less in its position paper in March 2018.[9]

Conclusions

The publication of these three landmark trials is a significant milestone in the quest for improved control of typhoid fever. More diverse efficacy and safety evaluation in immune-compromised hosts, children younger than 9 months and pregnant women will provide a more holistic picture of the potential usefulness of the TCV in routine immunisation practice.

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