Title: Innovative strategies for the surveillance, prevention, and management of pediatric infections applied to low-income settings.

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ABSTRACT

Introduction

Infectious diseases still cause a significant burden of morbidity and mortality among children in low- and middle-income countries (LMICs). There are ample opportunities for innovation in surveillance, prevention, and management, with the ultimate goal of improving survival.

Areas covered

This review discusses the current status in the use and development of innovative strategies for pediatric infectious diseases in LMICs by focusing on surveillance, diagnosis, prevention, and management. Topics covered are: Minimally Invasive Tissue Sampling as a technique to accurately ascertain the cause of death; Genetic Surveillance to trace the pathogen genomic diversity and emergence of resistance; Artificial Intelligence as a multidisciplinary tool; Portable non-invasive imaging methods; and Prognostic Biomarkers to triage and risk stratify pediatric patients.

Expert Opinion

To overcome the specific hurdles that child health in LMICs faces, some innovative strategies appear at the forefront of research. If the development of these next generation tools remains focused on accessibility, sustainability and capacity building, reshaping epidemiological surveillance, diagnosis, and treatment in LMICs, can become a reality and result in a significant public health impact. Their integration with existing healthcare infrastructures may revolutionize disease detection and surveillance, and improve child health and survival.

KEYWORDS

MITS; Bedside technology; Biomarkers; Child mortality; Infectious Diseases; Artificial Intelligence, Low-Income Countries; Pediatrics; Ultrasonography

ARTICLE HIGHLIGHTS

- The traditional research approaches in pediatric infectious diseases often face substantial barriers in low- and middle- income countries, such as scarce funding, limited infrastructure, lack of technical expertise, or logistical difficulties. This review discusses the current use and status of development of innovative strategies to overcome these hurdles.
- Minimally invasive tissue sampling, also known as minimally invasive autopsy, is a post-mortem technique to accurately ascertain the cause of death in children using fine needles to obtain samples from key organs, amenable to high-quality diagnostic techniques.
- Genetic surveillance involves developing international collaborative networks to trace pathogen genomic diversity and emergence of resistance in diseases such as malaria, tuberculosis, HIV, or COVID-19.
- Artificial intelligence is a multidisciplinary tool for imaging processing, epidemiological modelling, or language processing systems.
- Portable non-invasive imaging devices are attractive and accessible methods for screening and diagnosing malaria, tuberculosis, pneumonia, or meningitis.
- Host-response prognostic biomarkers related to endothelial and immune activation can be measured in point-of-care devices to triage and risk-stratify sick patients, and identify children at risk of dying.

ABBREVIATIONS

ABM: Acute Bacterial Meningitis.

AMR: Antimicrobial Resistance.

AI: Artificial Intelligence.

Angpt: Angiopoietin.

CDA: Complete diagnostic autopsy.

CoD: Causes of Death.

CHAMPS: Child Health and Mortality Prevention Surveillance.

CM: Cerebral Malaria.

CRP: C-Reactive Protein.

CSF: Cerebrospinal fluid.

CXR: Chest radiography.

IL: Interleukin.

LMICs: Low- and middle- income countries.

LP: Lumbar puncture.

LUS: Lung Ultrasound.

MDA: Mass Drug Administration.

MITS: Minimally invasive tissue sampling.

ML: Machine Learning.

PCT: Procalcitonin.

PoC: Point-of-care.

SEA: South-East Asia.

sFt-1: soluble FMS-like tyrosine kinase-1.

sTNFR-1: soluble tumor necrosis factor receptor 1.

sTREM-1: soluble triggering receptor expressed on myeloid cells 1.

SSA: Sub-Saharan Africa.

suPAR: Soluble Urokinase Plasminogen Activator Receptor.

REVIEW

1. INTRODUCTION

Pediatric infectious diseases still account for a significant burden of morbidity and mortality among children in low- and middle-income countries (LMICs). Resource-limited settings face specific challenges, including socioeconomic disparities and poverty, limited healthcare infrastructures and human resources, or inadequate access to preventive measures and healthcare services that exacerbate the impact of infectious diseases on pediatric populations. To address these challenges, conducting biomedical research is crucial for proposing new tools and interventions, deriving innovative and evidence-based strategies, and ultimately improve child health survival and clinical outcomes [1].

This article explores different approaches under development in surveillance, diagnosis, prevention, and management on LMICs for pediatric infectious diseases. Topics discussed on this review were chosen according to authors' criteria, not aiming to be a systematic analysis of literature. We have deliberately chosen to omit the topic of vaccines and climate change, given their demonstrated public health importance, but stand-alone importance.

2. SURVEILLANCE

2.1. Minimally Invasive Tissue Sampling (MITS)

Nearly 5 million of children under the age of five died worldwide in 2020, mainly in LMICs which now account for up to 99% of all child deaths [2]. In those areas where child mortality rates are highest, infectious diseases continue to be the most important causes of death (CoD), particularly in Sub-Saharan Africa (SSA) and South-East Asia (SEA), accounting for around 61.5% of all global child deaths [3]. Nowadays, the most common infectious causes of child mortality are believed to be pneumonia (15% of all under 5 deaths), diarrheal diseases (8.3%), malaria (5%), neonatal sepsis (7%), meningitis (2%), and measles (2%) [3]. However, this picture is far to be completely understood due to uncertainties and deficiencies in disease surveillance, death registries, and diagnostic

testing, especially in LMICs [4]. In those geographies, the main information for estimating CoD is based on verbal autopsy, and when available, on clinical records and death certificates. However, all of them have serious limitations and lack of reliability when compared with the complete diagnostic autopsy (CDA), which is considered the postmortem gold standard method for investigating a CoD [4]. In LMICs, the CDA is seldom performed, given to a variety of challenges that make it unfeasible and hardly accepted: starting with the lack of qualified staff and appropriate facilities to perform it; and followed by the existing social, cultural, and religious beliefs that typically hinder the acceptability of a rather disfiguring process, which may also cause delays in the typical burial practices. Consequently, there is a need to design and validate new robust and acceptable methods to ascertain CoD. Minimally invasive tissue sampling (MITS), also known as postmortem biopsy or minimally invasive autopsy, has emerged as a feasible and acceptable substitute of the CDA, providing comparable results, and is gaining major attraction.

MITS is a harmonized and well protocolized postmortem technique which uses biopsy needles to obtain targeted tissue samples from key organs (brain, heart, lungs and liver), body fluids (cerebrospinal fluid and blood), in addition to nasopharyngeal and rectal swabs without image guidance. These samples are then subject to comprehensive microbiology and pathology analyses [5]. The MITS approach in LMICs has been mainly developed and validated in the last decade, and has demonstrated a good level of concordance when compared against the CDA in all age groups and, interestingly, with higher rates of agreement for infectious diseases (68% to 89% of agreement between MITS and CDA) [6-8]. Most importantly, it has demonstrated a good acceptability by family members, and has been endorsed by healthcare workers [9]. Its feasibility is ensured by the fact that it can be performed by minimally-trained clinical staff, even in the absence of specific infrastructures or specialized pathologists. Such an approach has also been shown to be useful for investigating CoD in the context of outbreaks and epidemics, when full autopsies may be contraindicated [10,11].

Derived from the very encouraging results of the validation of the MITS approach, the Bill and Melinda Gates Foundation launched in 2016 the Child Health and Mortality Prevention Surveillance (CHAMPS) network, a long-term collaborative platform in different sites in SSA and Asia. CHAMPS has established a surveillance network in

which MITS technology is the core piece to collect, analyze, and share data. In addition to histopathological and microbiological information from body samples, clinical and demographic data are also collected, and verbal autopsies are conducted with family members. The final diagnosis is classified by a review panel of experts according to ICD-10 coding system [12]. Initial findings have revealed that infectious diseases continue to be the leading cause of death (neonatal sepsis in newborns [13]; or lower respiratory infection, diarrheal diseases, HIV, and malaria in children older than one month [14]). In comparison to previously utilized methods for determining the CoD in LMICs, MITS can give a more granular explanation on what caused the death of the child. Indeed, CHAMPS has highlighted the complexity of infections leading to deaths, often describing polymicrobial infections rather than single-organism problems. Organisms more commonly contributing to death have included: *Streptococcus agalactiae* (21%), *Escherichia coli* (16%), and *Enterococcus faecalis* (14%) in stillbirths [in press]; *Klebsiella pneumoniae* (45%), *Acinetobacter baumanii* (36%), and *Escherichia coli* (13%) in newborns [13]; or *Klebsiella pneumoniae* (28.2%), *Streptococcus pneumoniae* (19.9%), *Plasmodium falciparum* (22.2%), *Cytomegalovirus* (10.4%), and *Acinetobacter baumanii* (7.1%) among children 1-<60 months of age, noting that HIV was involved in 11.9% of deaths [15].

Importantly, CHAMPS data is changing our current understanding and the existing paradigms on what is causing childhood deaths in high-mortality settings, moving from a *one death-one cause* classical model to a more complex and multidimensional scenario in which most of the deaths may be explained by the concurrence of multiple causes, and where death appears as a concatenation of steps rather than a single event [13,14]. CHAMPS aspires to improve child survival by proposing evidence-based concrete measures to reduce mortality. It is important to highlight that 3 of 4 deaths in the network were deemed to have been preventable through improvements in antenatal and obstetric care, clinical management and quality of care, health-seeking behavior, and health education [16]. In following years, the accumulation of cases from CHAMPS sentinel sites will continue providing data to determine CoD in high-mortality areas, to inform policy, promote system and health interventions, and to prevent child mortality through a variety of tools, actions, and strategies.

2.2. Genetic Surveillance

Molecular epidemiology was born as a complement of classical epidemiology. Nowadays, genetic surveillance can help understand the genome diversity of pathogens; anticipate the appearance of new microbiological menaces [17]; and elucidate the source, timing, transmission, and spread of infectious diseases outbreaks [18]. Depicting genetic diversity is essential to perform optimal case traceability, identify transmission patterns, transmission chains, drug resistance markers identification, or detection of clusters of cases or the prevalence of given mutations among circulating pathogen populations [19,20]. The SARS-CoV-2 pandemic has been a recent and paradigmatic case [21]. Information enhanced by molecular analysis raised the importance of variant identification, allowing the monitoring of the impact of circulating variants on clinical outcomes, preventive and therapeutic needs, and wider epidemiological control [22]. In Mozambique, a recent study traced the origin and timing of SARS-CoV-2 variants nationwide: beta variants were mostly introduced from South Africa in August 2020, and delta variants from UK, India and South Africa in April 2021. The results suggest that movement restrictions effectively avoided introductions from non-African countries, but not from bordering countries [23]. The present aspiration would be to develop a real-time global pathogen surveillance system to enable rapid and effective responses to new epidemics. Next-generation sequencing platforms facilitates diagnostics through amplicon-based or metagenomics approaches, generating a stream of genomic data to reveal critical epidemiological aspects of an outbreak or epidemic's dynamics in just a few days [24]. Of particular interest in LMICs, *in situ* portable devices are being developed such as Illumina MiSeq system [25] or MiniION (Oxford Nanopore Technologies) [26], permitting sequencing in pocket devices that can be easily transported to remote regions, as they were used during the Ebola outbreak in West Africa [27].

Bacterial antimicrobial resistance (AMR) is a growing public health concern worldwide. In 2019, there were an estimated 4.95 million deaths associated with bacterial AMR, including 1.27 million deaths attributable to bacterial AMR in LMICs. The six leading pathogens for deaths associated with resistance were: *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* [28]. LMICs are particularly vulnerable to the spread of

AMR due to factors such as poor sanitation, inadequate healthcare infrastructure, and overuse of antibiotics. Different studies in several countries in SSA and Asia have reported a high rate of resistance to ampicillin (97.2%) and gentamycin (70.3%) bloodstream gram-negative isolates in neonatal sepsis, correlating with genotypes for cephalosporin and carbapenem resistance [29,30]; or 65% of enteropathogenic *E. coli* isolates in children under five years old with resistance to at least three or more drug classes, revealing a diversity of known genetic mechanisms for AMR that accounted for more than 95% of phenotypic resistance [31]. Other antibiotics like azithromycin have been used in Mass Drug Administration (MDA) programs, showing a reduction of all-cause mortality, and specifically for trachoma infection in children in LMICs. However, sparse evidence suggests that widespread and long-term exposure of children during MDA promote macrolide and other AMR [32,33].

The WHO considered that strengthen data on AMR through surveillance and research is one of the five strategic goals of the WHO global action plan, and highlighted that the lack of high-quality data and sustained funding limits the ability to assess and monitor trends of resistance worldwide [34]. In 2015, WHO launched the Global Antimicrobial Resistance and Use Surveillance System (GLASS) to standardize AMR surveillance in routinely collected samples, analysis, and sharing national data in collaboration with regional surveillance programs [35]. Although, currently, GLASS relies only on phenotypic resistance detection while in the future, genetic evaluation is expected to be introduced.

In the case of malaria, without a strong genetic surveillance cross-border system, we are blindfold for the appearance and spread of mutations that could impair the effectiveness of current treatments and control efforts [36]. Artemisinin combined therapies seem to be less effective in SEA, particularly in the Greater Mekong Sub-region, where partial artemisinin resistance and piperaquine resistance have been consistently reported since the first decade of 2000 [37]. Though this remains a localized problem, *de novo* emergence of partial artemisinin resistance has been reported from countries like Uganda and Rwanda [38,39]. MalariaGEN is an interdisciplinary network that integrate large amounts of data to study evolutionary processes that affect malaria transmission and disease (https://www.malariagen.net/) in an open dataset constantly updated, including the surveillance of emerging mutations worldwide [40]. Genomic surveillance can also

help monitor the outcomes of clinical and public health interventions. For instance, it can inform on genetic HRP2/3 deletions developed by *Plasmodium falciparum*, making this parasite 'invisible' to the most widely used diagnostic tests [41]. It can also help to track mutations on antimalarial compounds used in MDA campaigns or seasonal malaria chemoprevention [42] or new first-line treatments introduced in a region [43].

In the case of HIV, genomic surveillance in children in SSA detected a high rate of drug resistance mutations [44], including among drugs currently recommended in guidelines, therefore compromising therapeutic regimens [45]. Nevertheless, such methods also indicated the absence of integrase inhibitors resistance mutations, encouraging implementation of directed therapies [46]. In tuberculosis, some genomic signatures of pre-resistance can predict future antibiotic resistance and may enable targeted therapy to prevent its emergence [47,48].

To sum up, the implementation of a genomics-informed response to infectious diseases is an exciting possibility [18]. However, LMICs still face challenges for establishing routine genetic surveillance systems such as the elevated cost of the technology, lack of access to a reliable internet connection, lack of availability of consumables, supplies, reagents and maintenance, or limited staff capacity and training. To surpass those problems, collect reliable information and enhance preparedness for epidemiological challenges on pediatric infectious diseases, solid 'real-time monitoring' surveillance systems have to be established across countries.

3. PREVENTION AND DIAGNOSIS

3.1. Artificial Intelligence and Digital Health

The uses of artificial intelligence (AI) in medicine have been constantly growing in recent years. AI systems perform particularly well when used for tasks that are repetitive and prone to human mistake. Some applications are especially useful in LMICs, where accessibility to diagnostic tools, resources and specialized health workers are often limited [49]. AI-based technologies can notably help in the management of pediatric infectious diseases by, acquiring better epidemiological knowledge and building transmission models for predicting outbreaks, building clinical algorithms, improving

diagnosis (e.g. approaching specialized medical knowledge for case management and training in remote areas) and treatments (by reducing, for example, antimicrobial drug resistance or expanding drug indications) [50]

Epidemiological modeling facilitate the evaluation of different scenarios in variable epidemiological conditions and improving, among other applications, outbreak management, rapid decision making, and regional specific public health policies [51]. As a practical example, it has been used to evaluate the risk of COVID-19 spread and the impact of non-pharmaceutical interventions in refugee camps, which share common epidemiological characteristics with constrained-resource settings [51].

AI applications proved to be successful for a wide range of functions, including early-warning systems, hotspot detection, epidemiologic tracking and forecasting, and resource allocation [50]. Supervised Machine Learning (ML) algorithms are being used for risk assessment and prediction models to prioritize resources in LMICs. A multicentric study on children living with HIV and their progression to AIDS showed that innovative ML models, such as random forest, predicted the progression to AIDS with greater accuracy than traditional models like logistic regression [52].

Large language models (for example, Generative Pre-trained Transformer 4 [GPT-4]) hold the capacity for analyzing vast amounts of data and unstructured text coming from different sources (clinical records, social networks, or internet publicly available data) can identify epidemiological trends and detect disease outbreaks before traditional surveillance systems become aware [50]. While not specifically trained for healthcare purposes and still imperfect, there is also potential for their large-scale use in the near future in specific areas such as medical note taking, healthcare documentation, diagnosis, research, and education. These accessible tools can be particularly important in areas where the number of highly trained health-care professionals is limited [53].

As a diagnostic tool, AI has shown good performance for the analysis of medical images such as automatized electrocardiograms reading, or tuberculosis screening with chest radiography (CXR) or computed tomography scans [54]. This could be particularly useful in high-burden settings with lack of a sufficient number of radiologists to interpret radiological images. AI-based computer-aided detection that assess and interpret CXR

giving a probability for diagnosis can reduce inter-observer variability, mitigate clinician's workload, and provide a more accurate and homogeneous diagnosis. Available models in adults showed tuberculosis diagnostic performance with areas under curve ranging from 0.92 to 0.94 (95% CI 0.9-0.97) when compared to Xpert as a reference standard, and 0.85 to 0.92 (95% CI 0.82-0.93) compared to two sputum cultures [55]. These systems generally met the WHO's 90% sensitivity target profile product for screening. In fact, partially based on an individual patient data-level meta-analysis from four triage studies performed in Zambia, Tanzania, South Africa, and Pakistan [56], WHO recommended the use of computer-aided software for tuberculosis screening in CXR in patients over 15 years old [57]. However, these studies showed consistent lower sensitivity in specific subgroups such as people living with HIV, patients with smear-negative sputum, and patients with previous history of tuberculosis [56]. Pediatric patients are a complex population for AI-based computer-aided tuberculosis screening due to the absence of a reliable microbiological and composite reference standard for children with paucibacillary disease and a wide disease spectrum [55]. Current experimental programs are promising, but large external validation studies in heterogeneous populations are still needed to implement them on daily routine in a safely manner [54,58].

Developing point-of-care (PoC) ultrasound devices has been recognized as a global health priority [1], and some studies evaluating the performance in children are currently being done. A particularly interesting innovation is an AI-aided PoC for pediatric tuberculosis diagnosis. Ultrasound images are often difficult to interpret by humans, and AI systems can standardize the findings and reduce human observer misinterpretation of images [59]. In addition, in pediatric pneumonia, neural networks for automated diagnostic classification using ultrasound imaging of the lungs and pattern recognition showed a 90.9% sensitivity and 100% specificity [60].

Other applications include enhancing optical microscopy image reading. Convolutional Neural Networks for malaria parasites detection in blood slides can emulate the visualization of an expert microscopist with high yield, including microscope automation for a fast and low-cost diagnosis [61]. This is being developed not only for malaria but also for tuberculosis and other neglected tropical diseases [62]. As an example, using an adapter to put together the smartphone camera to a microscope, *Trichuris trichiura* (and

other helminths) in stool samples can be easily visualized. Images can be processed by a telemedicine platform assisted by deep learning, providing a diagnostic precision of 98.44% [63]. Moreover, AI-aided rapid diagnostic tests reading can improve the accuracy and speed of test results, reducing the cost and workload of healthcare professionals and interpretation´s subjectivity. For instance, it has been tested with excellent performance for reading cryptococcal antigen lateral flow assays with a simple smartphone app [64]. Taking step further, microscopy-based diagnosis (such as malaria or tuberculosis) can be gamified as a powerful tool for training. It was shown that exposing anonymous volunteers without prior experience to microscopic samples in a friendly-user mobile application (crowd gaming) could achieve excellent results for reading microscopy images in a very short period of time [61,65].

It is important to note that, despite clear advantages, some challenges in the development of AI remain unsolved, such as the scarcity of high-quality data, the applicability of existing models, different diagnostic performance patterns in specific population subgroups and comorbidities, or how these models are affected by cultural differences. AI may also pose important threats to global health, 'through increasing opportunities for control and manipulation of people, enhancing and dehumanizing lethal weapon capacity, and by rendering human labor increasingly obsolescent' [66]. International regulation and ethics assessment need to be considered in its development.

3.2. Portable noninvasive imaging devices

Pediatric infectious diseases pose challenges for health workers in LMICs due to unspecific symptoms and/or the overlapping signs in their clinical presentation, causing misdiagnoses, misallocation of resources, a poor use of antibiotics, and subsequent morbidity and mortality. In that context, there are many diseases that can directly benefit from technological solutions such as portable noninvasive imaging devices that can rapidly confirm or exclude determined life-threatening conditions next to patient's bed in resource-limited contexts. As representative examples, cerebral malaria (CM), meningitis, and pneumonia will be discussed subsequently.

Severe malaria is a complex multisystem disease that may present with multiple syndromes, including CM, the most lethal complication associated with a high risk of

death (~18%). CM is also associated with long-term cognitive and neurological deficits in up to 30-50% of survivors [67]. In consequence, a rapid and accurate diagnosis of CM could reduce its impact. Nonetheless, diagnosis of CM is not straightforward, and it is based on the exclusion of other diseases which may be part of its differential diagnosis. The pathognomonic changes observed in the postmortem evaluation of the brain microvasculature do have, however, an acceptable proxy in the retina, the only 'window to the brain' observable in an alive patient. Retinal screening serves as a valuable indicator of CM, with distinct malaria retinopathy highly suggestive of the diagnosis (retinal whitening, vessel abnormalities or discoloration, blot hemorrhages, papilledema, and cotton wool spots), which is also indicative of the severity of neurological injuries and prognosis of the patient [68]. The majority of health centers and hospitals in SSA lack specialized tools that could help visualize the retina, and as a result, there is very little practice on its observation by practicing clinicians. This could be solved with portable, affordable, user-friendly devices that allow a quick, intuitive, and direct observation of the fundus of the eye, permitting the recording of images and/or videos in a simple manner. Portable retinographs have been developed improving the issue of the difficult handling of traditional retinographs, also using smartphones as image acquisition and processing devices [69]. Interestingly, general practitioners without previous experience in the use of retinographs may easily use this device which can acquire clinically relevant data at the PoC [70].

Childhood pneumonia remains the first cause of death in children worldwide, despite the expansion of vaccination campaigns against some microorganisms like *Streptococcus pneumoniae* and *Haemophilus influenzae* type b [14]. The clinical diagnosis of pneumonia remains challenging for health staff in LMICs working with high patient volumes and without access to imaging techniques such as chest computed tomography, or, sometimes, even CXR. Lung ultrasound (LUS) is a promising pneumonia diagnostic technology with demonstrated sensitivity and specificity for the diagnosis of pneumonia, and greater inter-operator reliability than CXR [71]. In fact, LUS showed diagnostic performances of areas under curve ranging from 0.88 to 0.94 for radiographically-confirmed pediatric clinical pneumonia when compared to CXR [72,73]. Additional advantages of LUS, relative to CXR, include its portability, ease of use, lower cost, and absence of ionizing radiation. It can also be performed by non-specialized staff like nurses and clinical officers, even after a short and very basic training course, with a good

accuracy [72,74,75] and acceptance by health staff and caregivers [76]. LUS can complement CXR for pneumonia diagnosis when this is available, but can also be independently useful for diagnosis when there are no CXR facilities. However, further research is needed to standardize LUS patterns associated; to assess the potential use of serial ultrasounds in the clinical course of the disease; and to evaluate its sustainability and possible wider-scale implementation in the health systems of LMICs.

Meningitis is characterized by inflammation of the meninges. Some of them are caused by viral pathogens and have a benign course, but when they are caused by bacteria (e.g., *Streptococcus pneumoniae, Neisseria meningitidis)*, some viruses (e.g., herpesvirus…) or fungi (e.g., *Cryptococcus neoformans)*, they can be life threatening or cause long-lasting sequelae among survivors [77]. Despite all public health efforts, an estimated 2.5 million new cases of bacterial meningitis with 236,000 deaths worldwide still occur annually, the vast majority of which in LMICs [78]. Current diagnosis standards for meningitis involve performing a lumbar puncture (LP), an invasive procedure which is not exempt of risks and requires trained personnel and laboratory infrastructure, to obtain and to analyze cerebrospinal fluid (CSF). Resource limitations in LMICs hampers the process of appropriately performing and interpreting LPs. Consequently, the development of a simple, easy-to-use, non-invasive tool that could rapidly screen patients with acute febrile illness and confirm or discard the risk of acute bacterial meningitis (ABM) seems a global priority. In this respect, the not-yet closed fontanel in infants offers a unique window for the non-invasive early diagnosis of meningeal infections. Promising devices are currently under active development offering the opportunity of CSF cell counting through a non-invasive approach. High-frequency ultrasounds have shown in vitro capabilities to quantify cellularity in suspensions at concentrations as low as 10 cells per microliter [79]. Some preclinical studies even allow to differentiate between different cell types in fluid suspensions [80]. Animal studies with ultrasound frequencies equal or greater than 20 MHz show similar discrimination capabilities performance [79,81]. Current studies in human neonates with prototype devices are being conducted, and if validated, this tool may guide clinicians to discern which patients really need a LP from those who do not when screening for ABM, improving diagnosis and sparing potential harm and costs to the patient and the health system. Besides these non-invasive PoC devices, other approaches requiring invasive sampling can offer higher diagnostic precision and be feasibly implemented in LMICs, while meeting the PoC requirement of delivering timely

results at the patient's bedside. These tools naturally complement noninvasive systems and can integrate information for accurate diagnosis that guide patient's management and treatment. A clear example is the pilot Pediatric Infection-Point-of-Care (PI-POC) trial [82], which is evaluating the implementation of a novel DNA-based diagnostic assay for Central Nervous System infections, a commercially available multiplex PCR-based meningitis/encephalitis, and proteomic characteristics for central nervous system infection using blood, CSF, and nasopharyngeal swabs. Its validation will provide validated tools for PoC differential diagnosis and reduce irrational antimicrobial consumption.

3.3. Prognostic biomarkers

The epidemiology of febrile syndromes in LMICs remains poorly characterized. However, one of the main causes of clinical consultation is fever, especially in children, an age group in which it is -by far- the most common symptom at clinical presentation. The vast majority of febrile syndromes are uncomplicated and self-limited, but a small proportion (~1-2%) may progress to life-threatening infections. The early recognition of those children at greater risk of adverse outcomes is challenging due to the scarcity of available diagnostic and prognostic tools, and laboratory services, especially in rural and difficult-to-access areas [83]. The underlying problem is that the early identification of those children from a clinical standpoint is not straightforward, given that most cases of severe-to-become infections start with unspecific and overlapping symptoms. Thus, rapidly identifying children with a poor prognosis is very important for early prioritization, proper admission/referral, and adequate treatment provision [84].

For many years, the common approach was to prioritize accurate differentiation of the underlying etiology, assuming that identifying bacterial infections would prompt antibiotic treatment. However, many viral or parasitic infections may require the addition of empirical antibiotics due to the risk of associated invasive bacterial infections until co-infections can be ruled out, as is the case of severe malaria. Additionally, identifying a given pathogen is not a synonym of attributing causality of a certain disease. In this respect, prognostic tests (i.e., those tests informing on the associated risk of a patient progressing to a severe outcome, namely severe disease, death or severe sequelae among survivors) are gaining momentum for risk stratification and triaging practices. Prognostic

tests aim to predict the probability of clinical deterioration and fatal outcomes in sick patients upon arrival at a health facility, regardless of the diagnostic etiology. These tools could guide better management plans, referral practices, admission/discharge decisions, and therapeutic interventions, and serve, not as a substitute, but an optimal companion to etiology-based tests. Additionally, the development of PoC strategies for their measurements adds an important value when the turnaround time for results is reduced to minutes, enabling the clinician to make a fast decision [85].

Strong scientific evidence supports the feasibility of a strategy to identify such patients at higher risk. Many life-threatening infections like severe malaria, sepsis, pneumonia, or even COVID-19 share common pathways of injury related to the function and integrity of endothelium: immune and endothelial cell activation lay upstream of endothelial destabilization, microvascular leak, multi-organ dysfunction, and death [86,87]. Traditional biomarkers like C-Reactive Protein (CRP), Procalcitonin (PCT), lactate, lactoferrin, or leukocyte count showed acceptable performance for differential diagnosis between invasive bacterial and viral infections and are routinely used in high-income countries [88-90]. However, there are different markers of endothelial activation related to disease severity that seem to be superior to traditional biomarkers for predicting severity and death [91,92]. Some studies have shown that dysregulation of the Angiopoietin (Angpt)-Tie axis is associated with poor disease outcomes, in special high levels of Angpt-2 [93]. In addition, changes in plasma levels of different inflammation biomarkers such as Interleukin (IL-6), Interleukin (IL-8), soluble triggering receptor expressed on myeloid cells 1 (sTREM-1), soluble FMS-like tyrosine kinase-1 (sFt-1), soluble tumor necrosis factor receptor 1 (sTNFR-1), or Soluble Urokinase Plasminogen Activator Receptor (suPAR) have also been associated with severe disease outcomes [93,94].

Of this battery of biomarkers, sTREM-1 seems to be especially useful in febrile patients in LMICs and has been consistently associated with disease severity and mortality in many different contexts, and for most age groups including children [95]. sTREM-1 was the best prognostic marker for 28-day mortality with and area under curve of 0.87 (95% CI 0.81–0.92) and was significantly better than CRP ($p < 0.0001$) and PCT ($p = 0.0001$) in outpatient febrile adults [95]. sTREM-1 showed the best prognostic accuracy for 30-day intubation/mortality in PCR-confirmed COVID-19 patients, with an area under curve

of 0.86 (95% CI 0.77-0.95), significantly better than CRP 0.74 (95% CI 0.62-0.86) [96]. In children admitted with pneumonia, sTREM-1 was the best performing biomarker to predict poor prognosis (mortality and admission to intensive care) with an AUROC of 0.822 (95% CI 0.735-0.908), and significantly better than CRP, PCT, or white blood cell count [97,98]; and in a large cohort of febrile children, sTREM-1 at first clinical presentation had the best discrimination to predict 7-day mortality, with an AUROC of 0.893 (95% CI 0.84-0.94), superior to CRP, PCT, IL-8, and IL-6 [99].

Similar to sTREM-1, biomarkers can be added to clinical scores (such as Integrated Management of Childhood Illness [IMCI]) to enhance the management of febrile patients. Although these clinical scores have demonstrated an increase in the quality of care and have high sensitivity levels, they also have poor diagnostic specificity, particularly in young infants. There are some ongoing studies exploring the impact of such a combination both in diagnosis and prognosis in pediatric infectious diseases in LMICs [100]. Some near-patient strategies currently being explored using a combination of biomarkers, like the Rapid Acute Lung Injury Diagnostic (RALI-Dx), may also represent a valuable prognostic tool in the future [101].

Lastly, it's important to note that host-response biomarkers, easily measured in blood, not only inform on the prognosis but may also assist in selecting a more appropriate therapeutic approach based on the specific pathway affected.

4. CONCLUSIONS

Technological innovation in diseases that affect the most vulnerable populations in LMICs has enormous transformative potential to impact the significant burden they still cause in these settings. By embracing innovative approaches for surveillance, prevention, and treatment of pediatric infectious diseases, we can pave the way for effective interventions and timely diagnoses to reduce the burden of morbidity and mortality in LMICs. Thus, the integration of tools, such as PoC testing, molecular diagnostics, or AI, may enhance disease detection and surveillance, enabling us to anticipate diseases outbreaks or improve clinical outcomes. Nonetheless, these advances must be in harmony with more ‘traditional’ but essential public heath actions. We must continue working on

building sustainable research capacity, empowering local leadership, improving health infrastructure, and developing collaborative partnerships, which may promote strategic research for facing LMICs' structural and human capital limitations.

5. EXPERT OPINION

Traditional research approaches on the epidemiology, management, and prevention of pediatric infectious diseases often face substantial barriers in LMICs, such as scarce funding, limited infrastructure, lack of technical expertise, or logistical difficulties for consumable procurement. To overcome these hurdles, innovative strategies such as collaborative research networks, development of accessible noninvasive PoC diagnostic tools, or the use of AI and mobile technologies have emerged as powerful tools to foster impactful research in resource-constrained environments. Integration of these novel diagnostic technologies with existing healthcare infrastructure and the constant effort needed to reinforce public health systems has the potential to improve the control of pediatric infectious diseases, and to encourage local ownership and sustainability.

Some of the tools discussed in this article are not merely promises for the future but tangible realities. MITS has already been utilized in the context of surveillance efforts and research implementation studies and has the potential to become the methodology of choice in emergency situations such as infectious disease outbreaks. Further perspectives may require a careful evaluation for its wider implementation and integration into national surveillance programs and routine healthcare services.

Genetic surveillance has given an eye-opening perspective for drug resistance monitoring and public health strategies on prevention measures and treatment for many infectious diseases such as malaria, tuberculosis, and HIV. The fundamental hindrance for a wide implementation of genetic surveillance is related to the currently prohibitive costs, and the lack of expertise for sample processing and results interpretation. However, the development of suitcase sized portable devices, in parallel to capacity building improvement of infrastructures and personnel, can expand the access to these techniques. Its final aim would be to create a permanent, continuous, and real-time surveillance system that can raise awareness of health alerts.

AI has emerged as a ground-breaking technology affecting almost every discipline in human knowledge. Though many experts ask for prudence, rational, and consensual development of this technology due to its intrinsic risks, it is becoming evident that AI will become available and used to remodel our perspective of healthcare and research.

There are potential benefits on clinical management, training, research, or epidemiological surveillances for which we still do not know the real impact. Its use needs to be pushed to enhance the routine practice of clinicians in LMICs, always preserving the personal data protection rights and following ethical regulations.

Ultrasound is a powerful and hazard-free tool in pediatric infectious diseases imaging. In places where other diagnostic tools are scarce, it may help in the diagnosis of diseases like pneumonia and tuberculosis with portable and affordable devices. Although image interpretation is challenging for untrained eyes, this factor can be mitigated by AI-driven software that can support the analysis by giving a clinical actionable result.

Prognostic biomarkers are a promising tool that, if integrated with existing clinical algorithms, could help in the triage and risk-stratification of sick children. Such tools could provide actionable information to guide management, particularly relating to decisions on admission, discharge, or transfer to a higher care facility, irrespective of the underlying diagnosis. This 'agnostic' approach to infectious diseases could be important in remote areas where resources are scarce and healthcare workers training is limited, especially if such biomarkers can be used in PoC devices at the patient's bedside. The use of such markers to improve childhood survival, even so, still needs to be validated in well-designed randomized clinical trials.

In conclusion, all these advancements show potential in addressing the challenges faced by vulnerable pediatric populations in LMICs. Merging our knowledge and strategies to these new approaches and perspectives is required for overcoming problems inherent to those settings and take further steps for improving child health. Finally, we must not forget that technological innovation is not a substitute but a complement to those initiative aiming to reinforce public health systems; and that it must be accompanied by a biosocial perspective which consider that the impact of pediatric infectious diseases is driven not only by biological factors, but also by others of a historical, political, or sociocultural nature. Social research, political commitment, ethical discussion, legal assessment, and international regulations will be always necessary in the context of research and development.

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