PERSPECTIVE

Early Diagnosis of Febrile Illness: The Need of the Hour

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In clinical practice, children presenting with high grade fever of few days duration is a common scenario. The clinician is frequently faced with a situation where, clinical clues are subtle or minimal and a plethora of diagnostic modalities are available, and choosing the best option is a challenge. Herein, we briefly discuss the various rapid diagnostic tests (RDTs) or point of care tests (POC), available in the Indian scenario, that help elucidate the etiology of short duration fever in children.The significance of a detailed clinical history and physical examination cannot be overemphasized, and forms the basis for selecting from the battery of tests available.

THE NEED FOR EARLY DIAGNOSIS

Infectious diseases are responsible for an enormous burden of death and disability in developing countries, especially in children, thereby leading to a huge loss of healthy life-years [1]. Many people in developing countries do not have access to health care and laboratory facilities, and the diagnosis rests on the availability of RDT or POC tests [2]; so that treatment can be initiated at the earliest, to prevent complications and mortality.

Characteristics of an ideal POC test have been described as 'ASSURED' [3]:

Affordable; Sensitive; Specific; User-friendly (simple to perform in a few steps with minimal training); Robust and rapid (can be stored at room temperature and results available in <30 minutes); Equipment free or minimal equipment that can be solar-powered; and Deliverable to those who need them.

Epidemic dengue has spread to many new areas and has increased in the already affected South East Asia, which is home to 70% of the global at-risk population, with case fatality rates of 1-5% [4]. Typhoid fever continues to be a serious public health problem in many developing countries. It may lead to serious complications in 10-15% of cases with a case fatality rate of 1-4%. Global estimates range from 17 to 22 million cases per year and 216,000 to 600,000 deaths [5]. Half of the world's population is at risk of malaria, and as per WHO estimates, 243 million cases led to nearly 863,000 deaths in 2008 [6]. In India, around 1.5 million confirmed cases are reported annually by the National Vector Borne Disease Control Programme [NVBDCP], of which about 50% are due to *Plasmodium falciparum* [7].

All these conditions present diagnostic challenges as many clinical features are overlapping and non specific. There is no test available that can predict the progression of these illnesses to their life-threatening severe forms. Making the correct diagnosis is thus, crucial to prevent significant delay in starting appropriate therapy, reduce hospital stay and expenses, and prevent complications [8]. Early laboratory diagnosis is valuable, as some patients progress rapidly to severe disease and death, and also for surveillance activities, outbreak control, academic research, vaccine development, and clinical trials. The need for rapid diagnostic techniques has increasingly been felt to overcome this challenge. ***Table* 1** lists the various available RDTs.

DIAGNOSIS OF DENGUE FEVER

Dengue virus belongs to the family Flaviviridae, whose members share common cross-reactive antigens, complicating laboratory diagnosis. Virus isolation and PCR methods require sophisticated

TABLE 1: VARIOUS RDT'S AVAILABLE FOR DIAGNOSIS OF DENGUE, MALARIA AND ENTERIC FEVER

S. No.	Disease	Test	Type of Sample		Method Report	Time to of positivity	Earliest day of positivity	Latest day	Trade name (Rupees)	Cost	Sensitivity	Specificity
1	Dengue	NS1 Antigen	Serum	A	ICT	1 hour	1	9	NS1 (Panbio ICT)	775/-	70-97%	1
2	Dengue	Duo Ag-Ab	Serum	A	ELISA	Same day	1 (Ag)5(Ab)	9(Ag) 5 mths(Ab)	Dengue Ag-Ab Duo Rapid Screening Test	950/-	45-100%	57-100%
3	Dengue	IgM,IgG (qualitative)	Serum	A/R/F	ICT	Same day			Dengue IgM, IgG Qualitative test	1450/-		
4	Dengue	IgM (quantitative)	Serum	A/R/F	EIA	Same day	3-5 days	3 months	Dengue IgM	1350/-	83% (52-100%)	85% (53-99%)
5	Dengue	IgG (quantitative)	Serum	A/R/F	EIA	Same day	10-12 days	lifelong	Dengue Ig G	1350/-		
6	Enteric	Rapid IgM	Serum	A/R/F	ICT	Same day	3-5 days	2 weeks	Typhickeck, Typhidot	350/-	75%-92%	75-90%
7	Enteric	Widal	Serum	A	HA	24 hours	week2	3 weeks	Widal	300/-	40%-70%	60-75%
8	Malaria Heparin	Falciparum Ag	WB-EDTA/	A/R	Chromato-graphy	Same day	Anytime		Parachek F	525/-		
9	Malaria	Malaria Pan/Pf	WB-EDTA/ Heparin/ Citrate	A/R	ICT	Same day	Anytime		EZ Dx	600/-		
10	Malaria	IgG Ab	Serum	A/R/F	ICT	Same day			Malaria IgG	550/-		
11	Malaria	QBC	WB-EDTA	A	Fluore-scent microscopy	Same day	Anytime		QBC	475/-	75-96%	82-98.4%

Legend: NS - non structural, Ag – Antigen; Ab – Antibody; ICT – Immunochromatography; ELISA - Enzyme linked immunosorbent assay; WB- Whole blood; EIA - Enzyme immunoassay; Ig –Immunoglobulin; HA –Haemagglutination; A –Ambient; R –Refrigerated; F –Frozen.

laboratories, are expensive, and are not widely and easily available. Antibody-based tests [hemagglutination inhibition (HI) and IgM antibody capture ELISA (MAC-ELISA)] are approved for diagnosis of dengue infection. Both tests fail to discriminate between infections by other flaviviruses. The HI test is simple, sensitive, and reproducible but requires paired sera at least 1 week apart and thus is not very useful for clinical management. MAC-ELISA can measure a rise in dengue-specific IgM and IgG even in serum samples collected at 2-day intervals. This helps diagnose acute primary or secondary dengue infection. However, the need for proper timing of sample collection, false positive reactions, the long persistence of IgM antibodies, and limited availability are a few shortcomings [8].

In a recent meta-analysis of rapid (<60 minutes) diagnostic immunochromatographic test (ICT) for dengue, it was shown that the ICT can both rule in and rule out disease but is more accurate in samples collected in the late acute phase of infection [9]. The sensitivity of the ICT to differentiate between primary and secondary infection was suboptimal (66-71%) but the specificity, odds ratio and positive likelihood ratio indicated that it is an acceptable test for differentiating between the two [9].

Until recently, detection of dengue antigens in acute-phase serum was rare in patients with secondary infections because such patients had pre-existing virus-IgG antibody immune complexes. New developments in ELISA and dot blot assays directed to the envelop/membrane (E/M) antigen and the non-structural protein 1 (NS1) demonstrated that high concentrations of these antigens in the form of immune complexes could be detected in patients with both primary and secondary dengue infections up to nine days after the onset of illness. After day five, dengue virus and antigens disappear from the blood coincident with the appearance of specific antibodies. NS1 antigen may be detected in some patients for a few days after defervescence [4]. In a study by Kumarasamy, *et al*. [10], the dengue NS1 antigen-capture ELISA gave an overall sensitivity of 93.4% and a specificity of 100%. The sensitivity was significantly higher in acute primary dengue (97.3%) than in acute secondary dengue (70%). The positive predictive value of the dengue NS1 antigen-capture ELISA was 100% and negative predictive value was 97.3%. NS1 antigen ELISA was superior to virus isolation and RT-PCR for the laboratory diagnosis of acute dengue infection based on a single serum sample [10].

DIAGNOSIS OF ENTERIC FEVER

While the gold standard for definitive diagnosis of enteric fever is the bacteriological culture, the long time to availability of reports may limit its use. Widal test, though extensively used, cannot give a reliable diagnostic result in endemic regions due to difficulty in establishing a steady-state baseline titre, cross-reactivity with other organisms, effect of previous immunisation, inability to differentiate paratyphoid from typhoid, and lack of reproducibility of the result [11]. The timing of the widal test in a febrile illness is also important. It can give falsely positive results with other conditions such as malaria, immunological disorders and chronic liver diseases [12]. Even culture-positive typhoid patients may not produce detectable antibody levels, resulting in a false-negative serology [13,14].

Antibody-dependent tests [Multi-Test Dip-S-Ticks, TyphiDot, and TUBEX to detect IgG, IgG and IgM, and IgM, respectively] can be falsely-positive, particularly in endemic areas. Antigen-based tests become positive earlier in the illness before antibodies are identified or culture report becomes available. They can also help in the early detection of treatment failures and the carrier state. Narayanappa, *et al.* have reported that Typhidot-M was positive in 97% of cases who presented with fever of <7 days among blood culture positives as compared to Widal, which was positive in 24.2%, the overall sensitivity of the test was 92.6% [15]. In patients with fever >7 days duration, the IgM levels start declining and the IgG starts taking over, which can give rise to false negative results. Typhidot-M is easy to perform, sensitive, early, rapid [16], and requires minimal training, thus is an ideal screening test, though the higher cost is a limitation.

Enzyme immunoassays, counter-immune electrophoresis and co-agglutination tests to detect serum or urinary somatic/flagellar/Vi antigens of *Salmonella typhi* have also been evaluated. The suboptimal and

variable sensitivity and specificity estimates, inability to detect *Salmonella paratyphi* infection and Vi antigen negative strains of *S. typhi* are serious limitations of the Vi antigen detection tests [17]. The nested PCR-based diagnosis of typhoid could be a more useful tool than either blood culture or Widal test, owing to its greater discriminatory ability [18-20]. Case definitions based on combinations of serological tests can detect additional cases while maintaining 100% specificity [21].

With the sequencing of the entire serotype Typhi genome, it is possible to identify other antigens, such as fimbrial antigens, that may produce an antibody response specific to serotype Typhi [22].

DIAGNOSIS OF MALARIA

The increasing burden of the disease, the emergence of resistance to antimalarials, and availability of expensive artemesinin-combination therapies, especially in highly endemic regions, are increasing the need for rapid accurate diagnosis of patients with suspected malaria. WHO recommends that all case of fever clinically suspected as malaria should be confirmed either by microscopy or rapid diagnostic tests (RDTs) [6]. Despite being the "gold standard", the most important shortcoming of microscopic examination is its relatively low sensitivity, particularly at low parasite levels. The Quantitative buffy coat smear (QBC) technique is simple, reliable, and user-friendly, but it requires specialized instrumentation, is more expensive than conventional light microscopy, and is poor at determining the species and the number of parasites.

RDTs detect malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies (immunochromatographic lateral-flow-strip technology); they do not require laboratory equipment, are easy to perform and provide results within half an hour. Characteristics of a RDT vary based on regional malaria epidemiology and the goals of a malaria control programme [23]. The ideal test should be able to detect a response to therapy, and detection of recrudescence or relapse. Most products target a *P. falciparum*-specific protein, e.g. histidine-rich protein II (HRP-II) or lactate dehydrogenase (LDH). Some tests detect *P. falciparum* specific and pan-specific antigens (aldolase or panmalaria pLDH), and distinguish non-falciparum infections from mixed malaria infections. Despite their ability to discriminate between different species of malaria, the dipstick methods are poor at detecting mixed infections when one species is present at a significantly lower parasitemia than the other. The World Health Organisation (WHO) has recommended a minimal standard of 95% sensitivity for *P. falciparum* density of 100/μl, and a specificity of 95% [23,24]. Indian Academy of Pediatrics recommends the use of RDT'S in India in far away communities with poor access to health care facilities and non-availability of microscopic diagnosis; malaria in immunocompromised; in areas of multidrug resistance; and in severe and complicated cases [25].

The rapid diagnostic tests and microscopy can be utilized as complementary tools for maximum benefit; with RDTs providing a rapid or screening diagnosis, and microscopy reserved for resolution of confusing cases and verification of negative cases.

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