LABORATORY INVESTIGATIONS OF ENTERIC FEVER IN CHILDREN: AN UPDATE

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Abstract

Enteric fever is caused by salmonella group of organism. Prevalence of infection is high in developing countries. Little progress has been achieved in diagnosis of the condition in these areas. Presence of normal or low leukocyte count with esonopenia points to possible enteric fever. Blood culture remains effective investigation for diagnosis of enteric fever till date. Sensitivity of blood culture is highest in first week of illness. Bone marrow culture is a highly sensitive diagnostic test even in late disease despite prior antibiotic therapy. Culture of other materials e.g. stool, urine and rose spots on skin surface can be done. Nalidixic acid sensetivity test is essential to guide choice of antibiotics. Widal test has several limitations and should perform in second week of illness. This may be the only available test in poor setup for diagnosis of such condition. Slide Widal test is discouraged due to high rate of false positivity. Alternative serological tests e.g rapid dipstick assays, dot enzyme immuno-assays and agglutination inhibition tests may be carried out for rapid diagnosis of enteric fever. Validity of molicular diagnosis (PCR) is very high but the test is costly and requires sophisticated instruments.

Key words: enteric fever; investigation; children; blood culture; Widal test.

Introduction

Enteric fever is a systemic clinical syndrome producced by certain salmonella organism. It encompasses the terms typhoid fever, caused by salmonella typhi and paratyphoid fever caused by S. paratyphi A, S. schottmuelleri (formerly S. paratyphi B), S hirschfeldii (formerly S. paratyphi C) and occasionally other serotypes of salmonella. Though the incidence of this infection has decreased markedly in developed countries, in developing countries like ours the incidence is 0.5%. A complication of this condition involving intestine, heart, hepatobiliary system, lungs, kidneys, pancreas, and nervous system including intracranial

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complication is observed[1]. Despite its presence for a very long time, little progress has been observed in the diagnosis of the condition especially in developing countries. Diagnosis is till based on clinical features and on Widal test and very occasionally on blood culture. Many other tests are out of reach till dale. Important aspect is that many of our health personals are not fully aware of some important clinical aspects of such tests. This many lead to failure of diagnosis this condition or diagnose wrongly or very lately leading increase morbidity and mortality of our children. Parents of children may also face unnecessary anxiety and financial burden. Correct and rapid diagnosis of enteric fever is of paramount importance for instituting appropriate therapy and also for avoiding unnecessary therapy. The review is written to orient our health personals particularly clinicians regarding some fundamental aspects of laboratory investigations of such important issue so that diagnosis of enteric fever might be easier and appropriate, minimizing morbidity and mortality of our children from such infection.

Laboratory investigations

Complete Blood Count (CBC): For practical purposes CBC in enteric fever is unremarkable. Hemoglobin is normal in initial stage but drops with progressing illness. Severe anemia is unusual and should make one suspect intestinal hemorrhage or hemolysis or an alternative diagnosis like malaria. The WBC count is normal in most cases and leukocytosis makes the diagnosis less probable. Leukopenia has been reported in only 20-25% cases[2]. Dfferential count is usually unremarkable except for eosinopenia. Eosinopenia is often absolute and may be present in 70-80% cases[3]. Presence of absolute eosinopenia offers a clue for diagnosis but does not differentiate enteric fever from other acute bacterial or viral infections. Conversely, a normal eosinophil count does make typhoid fever a less likely possibility. Platelet counts are normal to begin with and fall in some cases by the second week of illness. Overall prevalence of thrombocytopenia is around 10-15%[4].

Cultures

Blood Culture: Blood culture is the gold standard diagnostic method for diagnosis of enteric fever[5].

Sensitivity of blood culture is highest in the first week of the illness and reduces with advancing diseases. Overall sensitivity is around 50% but drops considerably with prior antibiotic therapy[6]. Failure to isolate the organism may be caused by several factors which includes inadequate laboratory media, volume of blood taken for culture, presence of antibiotics and the time of sample collection. It is essential to inoculate media at the time of drawing blood. Salmonella can be easily cultured in routine culture media (Hartley's media, Blood agar and MacConkey agar). Sufficient amount of blood e.g at least 10 mL of blood in adults and 5 mL in children should be collected. Dilution should be appropriate in order to adequately neutralize the bactericidal effect of serum and a ratio of 1:5 to 1:10 of blood to broth is recommended. Sensitivity of clot culture, wherein the inhibitory effect of serum is obviated is not superior to blood culture[7]. Blood culture bottles should be incubated at 37°C and checked for turbidity, gas formation and other evidence of growth after 1, 2, 3 and 7 days. For days 1, 2 and 3 bottles only showing signs of positive growth are cultured on agar plates. On day 7 all bottles should be sub-cultured before being discarded as negative. There are considerable advantages of routine blood cultures in investigation of suspected enteric fever. They are not only 100% specific, but also provide information regarding antimicrobial sensitivity of the isolate. This is vital in present day scenario of multidrug resistance typhoid fever[8].

Bone marrow culture: Salmonella typhi is an intracellular pathogen in the reticuloendothelial cells of the body including bone marrow[5]. Studies have revealed that the median bacteremia in bone marrow is 9 CFU/mL compared to 0.3 CFU/mL in blood. This bone marrow: peripheral blood ratio which is around 4.8 in the first week of the illness increases to 158 during the third week owing to disappearance of bacteria from the peripheral blood[8]. The overall sensitivity of bone marrow cultures ranges from 80-95% and is good even in late disease and despite prior antibiotic therapy[5,9,10]. The invasive nature of bone marrow aspiration defers from its use as a first line investigation of enteric fever. It is however a very useful and valid test in evaluation of pyrexia of unknown origin(PUO) where in the marrow should be inoculated in the culture bottle at bed side[8].

Stool, urine and other cultures: Stool specimen should be collected in a sterile wide mouthed container. Specimens should preferably be processed within 2 hours after collection. If there is a delay the specimen should be stored in a refrigerator at 4°C or in a cool box with freezer packs. The sensitivity of stool culture depends on the amount of feces cultured, and the positivity rate increases with duration of the illness. Stool cultures are positive in 30% of patients with acute enteric fever. Rectal swabs should be avoided as these are less successful[5]. Several samples should be examined for detection of carriers because of irregular shedding of salmonella. Urine cultures are not recommended for diagnosis due to poor sensitivity[5]. Other methods such as duodenal string and skin snip culture of rose spots have been reported to be more efficacious than blood cultures but are mainly of academic importance[11].

Antimicrobial sensitivity testing: The crucial issue is to see the fluoroquinolone susceptibility testing. Fluoroquinolones were introduced in 1989 and during the past decade there has been a progressive increase in the MICs of ciprofloxacin in Salmonella typhi and paratyphi[5]. Since the current MIC's are still below the standard susceptibility breakpoint, laboratory reports will continue to report Salmonella typhi/paratyphi as ciprofloxacin/ofloxacin sensitive[12]. However, use of fluoroquinolones in this scenario is associated with a high incidence of clinical failure[5,12]. It has also been demonstrated that resistance to nalidixic acid is a surrogate marker for high ciprofloxacin MIC's, predicts fluoroquinolone failure and can hence be used to guide antibiotic therapy. If culture results show resistance to nalidixic acid irrespective of the results of quinolones sensitivity, quinolones should not be used or if used high doses should be given[13]. Since MIC testing is not within the scope of most laboratories, nalidixic acid susceptibility testing is mandatory to guide to choice antibiotics.

Serologic tests: Widal test: This test first described by F Widal in 1896, detects agglutinating antibodies against O and H antigens of Salmonella typhi and H antigens of paratyphi A and B[6,14]. The "O" antigen is the somatic antigen of Salmonella typhi and is shared by Salmonella paratyphi A, paratyphi B, other Salmonella species and other members of the Enterobacteriaceae family[15]. Antibodies against O antigen are predominantly IgM, rise early in the illness and disappear early[15]. The H antigens are flagellar antigens of Salmonella typhi, paratyphi A and paratyphi B. Antibodies to H antigens are both IgM and IgG, rise late in the

illness and persist for a longer time[14,15]. Usually, O antibodies appear on day 6-8 and H antibodies on days 10-12 after the onset of disease[8]. Conventionally, a positive Widal test result implies demonstration of rising titers in paired blood samples 10-14 days apart[14]. Unfortunately, this criterion is purely of academic interest. Decisions about antibiotic therapy cannot wait for results from two samples. Moreover, antibiotics may dampen the immune response and prevent a rise in titers even in truly infected individuals. Therapeutic decisions have to be generally based on results of a single acute sample. In endemic areas, baseline anti O and anti H antibodies are present in the population owing to repeated subclinical infections with Salmonella typhi/paratyphi, infections with other Entero-bacteriaceae and other tropical diseases such as dengue and malaria[14-16]. These antibody titers vary with age, socio-economic strata, urban or rural areas and prior immunization with TAB vaccine. Establishing appropriate cut offs for distinguishing acute from past infections is thus important for population where the test is applied. Study from Central India showed that anti O and anti H titer of more than 1:80 was seen in 14% and 8% respectively of a sample of 1200 healthy blood donors[8].

Both H and O antibodies have to be taken into account during interpretation of the Widal test. Controversy regarding predictive value of O and H antibodies for diagnosis of enteric fever is existing. Somebody claim that O antibodies have superior specificity and positive predictive value because these antibodies decline early after an acute infection[8]. Other report a poorer positive predictive value of O antibodies probably due to rise of these antibodies in other salmonella species, gram-negative infections, in unrelated infection and following TAB vaccination[16]. Practrically,this test should be done after 5-7 days of fever by tube method and level of both H and O antibodies of 1 in 160 dilution (four fold rise) should be taken as cut off value for diagnosis. H antibodies once positive can remain positive for a long time[8].

The Widal test as a diagnostic modality has suboptimal sensitivity and specificity[14-16]. It may be negative in up to 30% of culture proven cases of typhoid fever. Sub optimal sensitivity results from negativity in early infection, prior antibiotic therapy and failure to mount an immune response by certain individuals[14]. Poor specificity is a consequence of pre-existing baseline antibodies in endemic areas, cross reactivity with other gram-negative infections and non-typhoidal salmonella, anamnestic reactions in unrelated infections and prior TAB or oral typhoid vaccination. The slide Widal test should be discouraged owing to high rate of false positives[15]. Till that, the Widal test may be the only diagnostic test available in certain centers. In Vietnam, using a cutoff of >1/200 for the O agglutinin or >1/100 for H agglutinin test performed on acute-phase serum the Widal test could correctly diagnose 74% of blood culture positive typhoid fever, however 14% results would be false positive and 10% false negative[16]. Hence, Widal test should be carefully interpretated to overcome both over and under diagnosis of typhoid fever[8].

Other serologic tests: Due to limitations of the Widal test and need for a cheap and rapid diagnostic method, several attempts have been made to develop alternative serologic tests. These include rapid dipstick assays, dot enzyme immuno-assays and agglutination inhibition tests[17-19].

Enzyme Immunoassay (EIA) test or Typhidotâ test: This test detects IgG and IgM antibodies against a 50 KD outer membrane protein distinct from the somatic (O), flagellar (H) or capsular (Vi) antigen of Salmonella typhi[19]. Sensitivity and specificity of this test has been reported to vary from 70-100% and 43-90% respectively[20-23]. This dot EIA test offers simplicity,early diagnosis and high negative and positive predictive values. Detection of IgM reveals acute typhoid in early phase of infection, while detection of both IgG and IgM suggests acute typhoid in the middle phase of infection. After typhoid infection IgG can persist for more that 2 years. So detection of specific IgG can not differentiate between acute and convalescent cases[24]. Moreover, false positive results may occur attributable to previous infection. On the other hand IgG positivity may also occur in the event of current reinfection. In cases of reinfection there is a secondary immune response with a significant boosting of IgG over IgM, such that the later can not be detected and its effect masked. A possible strategy for solving this problem is to enable the detection of IgM by ensuring that it is unmasked[25]. The original Typhidotâ test was modified by inactivating total IgG in the serum sample. In modified Typhidotâ M, it has shown that inactivation of IgG removes competitive binding and allows the access of the antigen to the specific IgM

when it is present. The Typhidotâ M that detects only IgM antibodies of Salmonella typhi has been reported to be slightly more specific in a couple of studies[18,23].

IDL Tubexâ test: The Tubexâ test is easy to perform and takes approximately 2 minutes time[26]. The test is based on detecting antibodies to a single antigen in S. typhi only. The 09 antigen used in this test is very specific found in only sero group D salmonellae. A positive result always suggest a salmonellae infection but not which group D salmonella is responsible. Infection by other serotypes like S. paratyphi A give negative result. This test detects IgM antibodies but not IgG which is further helpful in the diagnosis of current infections[8]. IgM dipstick test: This test is based on binding of S. typhi specific IgM antibodies to S. typhi lipopolysaccharide (LPS) antigen and the staining of the bound antibodies by an antihuman IgM antibody conjugated to colloidal dye particles. This is an easy, simplified test and may be a useful particularly where culture facilities are not available. One should keep in mind that specific antibodies appear a week after the onset of symptoms[18]. Antigen detection tests: Enzyme immuno-assay's, countcrimmune electrophoresis and co-agglutination tests to detect serum or urinary somatic/flagellar/Vi antigens of Salmonella typhi have been evaluated[27,28]. Sensitivity of Vi antigen has been found to be superior to somatic and flagellar antigen and ranges from 50-100%[27,28]. Specificity of Vi antigen detection test is 25-90%[27,28q]. Suboptimal and variable validity of this test to detect Salmonella paratyphi infection and Vi antigen negative strains of S typhi is a great limitation of Vi antigen detection test[8].

Molecular methods
The limitations of cultures and serologic tests advocate for development of alternative diagnostic strategies. Polymarease chain reaction (PCR) as a diagnostic modality for typhoid fever was first evaluated in 1993 through amplification of flagellin gene of S. typhi in all cases of culture proven typhoid fever and in none of the healthy controls8. Moreover, some patients with culture ncgative typhoid fever were PCR positive suggesting that PCR diagnosis of typhoid may have superior sensitivity than cultures. Over the next 10 years a handful of studies reported validity of PCR methods targeting the flagellin gene, somatic gene, Vi antigen gene, 5S-23S spacer region of the ribosomal RNA gene, invA gene and hilA gene of Salmonella typhi

for diagnosis of typhoid fever[29-33]. These studies have reported excellent sensitivity and specificity when compared to blood culture positive cases and healthy controls. The time needed for such valid method is less than 24 hours[8].

The clinical utility of PCR tests has been inadequately evaluated. Performance of the test in individuals with febrile illnesses other than typhoid, in those with past history of typhoid, carriers of S typhi, and those vaccinated with typhoid vaccine is not known. Patients with a clinical diagnosis of typhoid fever who are culture negative but PCR positive may in fact be false positives. Comparison of PCR to bone marrow cultures as a gold standard may be a superior way of evaluating the sensitivity and specificity of these tests, but has not been done. The tests claim to detect as few as 10 organisms8. Using small volumes of blood for DNA extraction may significantly lower the sensitivity of these tests. The cost and requirement of sophisticated instruments is a great limitation of molecular diagnosis of enteric fever.

Messages

> *Esonopenia may be present in 70-80% cases of enteric fever. Presence of normal or low leukocyte count with esonopenia points to possible enteric fever.
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> *Blood culture is the gold standard for diagnosis of typhoid fever. A positive culture unequivocally establishes the diagnosis and gives the sensitivity pattern.
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> *Nalidixic acid sensitivity is a surrogate marker of fluoroquinolone sensitivity and nalidixic acid susceptibility testing is essential to guide the choice of antibiotics.
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> *Sensitivity of marrow culture is 80-95 % even in late disease and prior to antibiotic therapy. Marrow should be inoculated in the culture bottle at bed side.
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> *Widal test has poor sensitivity due to its negativity in early infection, prior antibiotic therapy and poor immune response of certain children. It should be done after 5-7 days of fever by tube method and level of 1 in 160 for both H and O antibodies are usually taken as diagnostic.
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> *Typhidot test detects IgG and IgM against outer membrane protein of S. typhi. As IgG can persist over a long time it is difficult to distinguish between acute infection and convalescent stage. This is improved in modified typhidot M test which detects only IgM antibodies.

Conclusion

Complete blood count is the first investigation for diagnosis of enteric fever. Presence of a normal or low leukocyte count with eosinopenia points to possible enteric fever. This also helps in evaluation of alternative diagnosis such as malaria, dengue and other bacteremias. Blood culture remains the most effective investigation for diagnosis of enteric fever till date. They should be sent early in the course of the illness and prior to starting antibiotic therapy. Susceptibility testing for nalidixic acid should be routinely done for all isolates to aid choice of antibiotics. Bone marrow culture is a highly sensitive diagnostic test even in late stages of the illness and with prior antibiotic therapy. It should be performed in all patients with prolonged pyrexia if routine investigations have failed to establish a diagnosis. Widal test has several limitations; should be done in second week of illness and results should be interpreted cautiously. Other newer serological tests are also available and the modified typhidot M test detects only recent infection. The molecular diagnosis of enteric fever is promising but expensive and high technology is needed.

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