The TUBEX typhoid test detects current Salmonella infections

Article in Journal of Immunological Methods · March 2004
DOI: 10.1016/j.jim.2003.10.020 · Source: PubMed

5 authors, including:

Wojciech Feleszko
Medical University of Warsaw
69 PUBLICATIONS 1,222 CITATIONS

Some of the authors of this publication are also working on these related projects:

Contact allergens in topical corticosteroid vehicles View project
Rapid Diagnosis of Acute Salmonella Gastrointestinal Infection

Grzegorz Oracz,1*ab Wojciech Feleszko,1* Dorota Golicka,7 Joanna Maksymiuk,2 Agnieszka Klonowska,1 and Hanna Szajewska1

Departments of aPediatric Pneumonology, Allergy, and Hematology and bPediatric Gastroenterology and Nutrition, cMedical University of Warsaw, Warsaw, Poland

Serologic tests for the detection of Salmonella serotype Enteritidis in children may become supplementary to stool culture examination. A total of 190 children were examined with a new 1-step, 2-minute test (TUBEX) that detects anti-Salmonella immunoglobulin M antibodies, which was found to be 92.6% sensitive and 94.8% specific (P<.0001).

Acute infectious diarrhea is a common disease in childhood, and Salmonella is the most likely bacterial causes in children in industrialized countries [1, 2]. The reported incidence of salmonellosis has increased during the past 50 years in the United States and remains an important cause of diarrheal illness, causing ∼1.4 million episodes and 600 deaths annually [3, 4]. Food-borne, non-Typhi serotypes, mainly group D Salmonella (Salmonella enterica subspecies enterica serotype Enteritidis) predominate among the most common Salmonella serotypes, with the highest incidence rates among infants aged <1 year and a definitely high frequency among children aged <9 years [2]. The dominance of only few serotypes is more pronounced in the European countries. For example, in Poland in 1999, the most frequent serotype, Salmonella serotype Enteritidis, was identified in ∼92% of 23,000 cases [5], thus remaining a leading cause of bacterial infectious diarrhea. Salmonellosis in older children and adults is usually a self-limited disease (presenting as acute gastroenteritis), and therapy should mainly be directed at preventing dehydration [1, 6]. However, it is justified to use antimicrobial therapy in infants aged ≤3 months with Salmonella gastroenteritis, as well as in immunocompromised patients and patients with septicemia [1, 6, 7]. In these patients, antibiotic treatment will be the most successful in the early stages of illness, and delaying treatment may result in septicemia-related dehydration and renal failure. Because the early stage is often clinically difficult to determine (because stool and blood culture results are generally available in ∼2 days), these patients might benefit from serologic tests that can be done quickly in the place where the patient is receiving care.

Lim et al. [8] reported the discovery of a new, quick serologic test for detecting S. Typhi, in which anti-Salmonella O9 IgM antibodies are detected, and this test seems to be more rapid and accurate than the Widal agglutination test. Both S. Typhi and group D Salmonella display similarities in their O9 antigen, which contains a sugar, α-d-tyvelose, that is extremely rare in nature [9]; therefore one cannot dismiss the notion that this test may also be useful for the serodiagnosis of nontyphoid salmonellosis. In this study, we aimed to assess the accuracy of the 1-step, 2-min test (TUBEX; IDL Biotech), compared with that of routine stool culture examination.

Patients and methods. We recruited children from 2 pediatric hospitals in Warsaw, Poland, from April 2000 to January 2002. All consecutive children aged <16 years who were admitted to the hospital for acute diarrhea (defined as ≥3 profuse, watery, or mucous stools for >1 day but <5 days in patients who required intravenous rehydration) were eligible for the study (figure 1). Children who had a documented history of salmonellosis in the past, who had primary or secondary immune deficiencies, or who were receiving systemic hormonal treatment were excluded. The control group consisted of children aged <16 years who were admitted for acute diarrhea, but whose stool cultures were negative for Salmonella species. Informed consent of parents was obtained in each case, and human experimentation guidelines of the Medical University of Warsaw were followed in the conduct of this clinical research.

For all patients, stool samples were collected at admission and were inoculated onto Salmonella-Shigella and McConkey agar (Centralna Pozwarkarnia SanEpid) in the laboratory within 2 h of collection. Organisms were identified by standard methods, including triple-sugar iron slants, and were serotyped with commercial serotyping antiserum (Biomed). Patients then were assigned to one of the experimental groups, and their blood
samples (drawn as part of routine laboratory examination) were collected and stored frozen at $-20\,^\circ\mathrm{C}$ until analysis.

The serodiagnosis of infection with *S. Enteritidis* was performed by means of the TUBEX test, which detected anti-*Salmonella* O9 IgM antibodies in the serum, as described elsewhere [8]. In brief, the inhibition of binding between an anti-*Salmonella* O9 IgM monoclonal antibody conjugated to colored latex particles and *S. Typhi* lipopolysaccharide conjugated to magnetic latex particles was measured, and the resultant color of the supernatant was recorded in a blinded fashion by 2 investigators and then was compared with color standards provided by the manufacturer. Next, an appropriate score between 0 and 10 was assigned. A TUBEX score of 4, corresponding to detection of 31 micrograms of the anti-*Salmonella* antibody per milliliter, was arbitrarily taken as the lower limit for a positive reaction [8].

For the evaluation of the results of this study, a contingency table analysis was performed (calculating the 95% CI values) and was computed by Instat 2.0 software (GraphPad Software). $P < .05$ was considered to be statistically significant.

**Results.** One hundred ninety patients (94 in the study group and 96 in the control group) were enrolled in the study from April 2000 to January 2002 (figure 1). Eighty-seven (92.6%) of 94 children infected with *S. Enteritidis* had a positive TUBEX result. The positive scores obtained ranged from 4 (23 patients) to 10 (27 patients; figure 2). The median score was 7 (95% CI, 6.82–7.1). In contrast, 91 (94.8%) of 96 control serum samples had a score $< 4$ (a negative TUBEX result), with a median score of 0 (95% CI, 0.2–0.7; figure 2). These patients corresponded in age and sex with those in the examined group.

No correlation between antibody concentration (expressed as TUBEX score) and duration of diarrhea in *Salmonella*-infected patients was observed.

The difference in the TUBEX score between *Salmonella*-infected patients and the control subjects was highly significant ($P < .0001$, Mann-Whitney $U$ test, 2-tailed). Compared with that of the stool culture, the sensitivity and specificity of the TUBEX test were 92.6% (95% CI, 85.3–97.0) and 94.8% (95% CI, 88.3–98.3), respectively; the positive and negative predictive values were 94.7% (95% CI, 87.7–98.2) and 92.9% (95% CI, 85.8–97.1), respectively. Statistical evaluation of the results revealed that the row/column association was extremely significant ($P < .0001$, Fisher’s exact test, 1-sided). The positive likelihood ratio calculated for this test was 17.7, and the negative likelihood ratio was 0.079.

**Discussion.** In this study of 190 children, the new serologic test was proved to be effective in the serodiagnosis of non-typhoid salmonellosis. This test was primarily designed to di-
agnose typhoid fever and reportedly was 100% sensitive and specific in the detection of S. Typhi infection [8]; however, in our study, a 92.6% sensitivity and 94.8% specificity for non-typhoid salmonellosis was demonstrated. These results correspond significantly to the results of stool culture. Although microbiological examination of blood or fecal samples remains the “gold standard” laboratory test for the confirmation of salmonellosis, a rapid serologic test based on antibody detection may provide a convenient supplementation. The value of the old serologic diagnosis (with the Widal agglutination test) is often questioned [10], and the most sensitive and specific assay, ELISA, is prohibitively expensive [8, 10]. For these reasons, a sensitive, simple, and cost-effective serologic diagnostic method for the diagnosis of Salmonella infection may result not only in rapid instigation of salmonellosis control measures but also in prompt diagnosis followed by appropriate treatment.

S. Enteritidis remains the most likely bacterial cause of foodborne, acute gastroenteritis in industrialized European countries; the other serotype, group D Salmonella, seems to not play any significant role [5, 11]. Only sporadic cases of typhoid fever are registered in the European countries and the United States [11], and no single case of S. Typhi was observed in Poland in the past 10 years [5]. Our results highlight the potential of the TUBEX test in the supplementary diagnosis of acute infectious gastroenteritis in these regions; however, its applicability for diagnosis of typhoid fever in these countries is limited. In view of these facts, it seems unlikely that positive results of the test reflected false-positive findings, possibly due to preexisting S. Typhi infections. However, the problem of false-positive results due to previous S. Enteritidis infections remains to be investigated. In this study, the serodiagnosis of Salmonella infection was performed within 3–8 days after the onset of the symptoms, which might positively affect the level of sensitivity of the test. This requires further investigation.

In conclusion, our data highlight the potential of the TUBEX test to supplement existing methods of diagnosis of Salmonella infections. TUBEX has been demonstrated to be a rapid, cost-effective, simple, and sensitive method for the diagnosis of Salmonella infection, and its positive likelihood ratio in our study was relatively high (17.7). One may speculate that this test also may be applied to individuals with a postinfectious reactive arthritis, for whom stool culture results are often negative [12]. To our knowledge, this report is the first to demonstrate the potent diagnostic value of quick diagnosis of S. Enteritidis–derived gastrointestinal infection; however, further studies are warranted for evaluation of the clinical applicability of TUBEX as a novel approach for the diagnosis of Salmonella infection.

References