JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1983, p. 872–876
0095-1137/83/100872-05$02.00/0

Vol. 18, No. 4

Detection of Urinary Vi Antigen as a Diagnostic Test for Typhoid Fever

DAVID N. TAYLOR,[1]†* JEFFREY R. HARRIS,[1]‡ TIMOTHY J. BARRETT,[1] NANCY T. HARGRETT,[1] INGEBORG PRENTZEL,[2] CARMEN VALDIVIESO,[2] CARMEN PALOMINO,[3] MYRON M. LEVINE,[4] AND PAUL A. BLAKE[1]

Enteric Diseases Branch, Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333[1]; Department of Microbiology, Roberto del Rio Hospital, and Hospital de Enfermedades Infecciosas,[3] Santiago, Chile; and Center for Vaccine Development, University of Maryland, Baltimore, Maryland 21201[4]

Received 24 March 1983/Accepted 20 July 1983

Since Vi antigen is limited primarily to *Salmonella typhi*, it has been thought that detection of the antigen may be a useful method for diagnosing acute typhoid fever. The slide coagglutination method and enzyme-linked immunosorbent assay have recently been suggested as ways to detect small quantities of Vi antigen in urine. In Santiago, Chile, we compared the results of these two methods in patients with acute typhoid fever, paratyphoid fever, and other febrile illnesses and in afebrile control subjects. Using a cut-off value that maximally separated typhoid patients from controls, the enzyme-linked immunosorbent assay was positive in 62.4% of 141 patients with culture-proven typhoid infections and in 13.2% of 159 afebrile control subjects. The enzyme-linked immunosorbent assay was false positive in 64.7% of 34 culture-proven paratyphoid A or B patients and 47.1% of 21 patients with other nontyphoidal febrile illnesses. The coagglutination test was positive in 34% of typhoid patients, 14% of afebrile control subjects, and 46% of febrile control subjects. We conclude that these tests when performed with the Vi antibodies employed in this study are of little value for the diagnosis of typhoid fever in this setting.

Microbiological methods for the diagnosis of typhoid fever are frequently not available in rural areas of developing countries, where it is a major problem. When microbiological methods are available, identification of *Salmonella typhi* from blood or stool often takes several days and can be falsely negative if antimicrobial agents have been previously administered. Currently used immunological methods to diagnose typhoid fever are of limited value. The most widely used immunological assay is the Widal test for serum antibodies to O and H antigens. In areas of the world where typhoid fever is endemic, the test has been found to be sensitive but lacking in specificity. For example, Levine et al. have demonstrated O antibodies to be elevated in 90% of typhoid patients in Mexico but found O and H antibodies elevated in 30 and 75%, respectively, of healthy Peruvians (4). Antibodies to the capsular Vi antigen of *S. typhi* are more specific for typhoid fever but appear too late in the course of illness to affect decisions on therapy (8).

Attempts to improve immunological methods have focused on Vi antigen detection since this antigen is limited primarily to *S. typhi* and would presumably be present early in the course of infection. Rockhill et al. have reported a slide coagglutination test to detect soluble *S. typhi* Vi, D, and d antigens in the urine of Indonesian patients with typhoid (7). In a group of 61 culture-confirmed patients and 46 well control subjects, the test was 97% sensitive and 83% specific. To improve sensitivity and specificity, Barrett et al. used an enzyme-linked immunosorbent assay (ELISA) to detect urinary Vi antigen (1). The ELISA provided an increase in both sensitivity and specificity over the slide coagglutination method in laboratory tests with pure antigen and in a limited field trial performed in our laboratory.

Typhoid fever is endemic in many areas of South America. In Santiago, Chile, the annual incidence is 90 cases per 100,000 inhabitants. Over 50% of the cases occur during the summer months (December through March), and approximately 65% of the cases occur among persons 5 to 19 years of age (6). We compared the diagnos-

† Present address: U.S.A. Medical Component, Armed Forces Research Institute in the Medical Sciences, APO San Francisco, CA 96346.

‡ Present address: State Department of Health and Social Services, Seattle, WA 98104.

tic capabilities of the coagglutination test and the ELISA in Santiago because of the particularly high incidence of typhoid, the availability of a well-equipped laboratory, and the current interest in typhoid fever stimulated there by an ongoing oral typhoid vaccine trial.

MATERIALS AND METHODS

Case selection. Blood, stool, and occasionally marrow cultures are routinely obtained from patients seen at a large municipal pediatric hospital (Roberto del Rio Hospital) or at an adult infectious diseases hospital (Infectious Diseases Hospital) in Santiago, Chile, when the diagnosis of typhoid or paratyphoid fever is considered. We collected urine specimens (15 ml) from patients with suspected typhoid fever seen at these two hospitals during January and February 1982. Patients with suspected typhoid fever were interviewed, and their hospital records were reviewed, to ascertain age, sex, hospital, date of admission, date of onset of fever, typhoid history, typhoid vaccination history, symptoms, physical findings, and antimicrobial treatment. A case of typhoid or paratyphoid was defined as the isolation of *S. typhi* or *Salmonella paratyphi* from blood, stool, or bone marrow from a patient clinically suspected of having acute typhoid fever.

Selection of controls. Afebrile patients admitted to surgical wards of Roberto del Rio Hospital or to a general medical hospital (Trudeau Hospital) adjacent to the Infectious Diseases Hospital during January and February 1982 were selected as afebrile control subjects. These patients were usually admitted for elective surgery and had no history of recent infections. The reason for admission, history of previous typhoid, and typhoid vaccination history were recorded. Patients who were admitted to the pediatric or the adult infectious diseases hospital who had a clearly diagnosed, nontyphoidal febrile illness were selected as febrile control subjects.

Specimen handling. Urine specimens were collected from all inpatients during the first 2 days after admission and from outpatients at the time blood cultures were obtained. The urine was collected in 15-ml plastic tubes, refrigerated at 5°C overnight, and tested for the presence of Vi antigen the next day.

Microbiological methods. Five milliliters of whole blood was inoculated into 50 ml of brain heart infusion broth at the pediatric hospital, and 2 ml of whole blood was inoculated into 15 ml of tryptic-salt-glucose broth at the infectious diseases hospital. Stool specimens were inoculated onto a solid agar medium (S-S or MacConkey). *S. typhi* and *S. paratyphi* were identified by standard methods (2).

Immunological methods. Serum specimens obtained at admission from patients with febrile illnesses were processed at each hospital for O and H antigens (Widal test) by standard methods. Vi antibody titers were determined in 24 paired acute-phase and convalescent-phase serum samples obtained during the course of the study from typhoid patients by an indirect hemagglutination method (5).

The slide coagglutination test was done by the method of Rockhill et al. (7), with Cowan I *Staphylococcus aureus* cells coated with anti-*Citrobacter* Vi rabbit serum (1). Control cells were coated with normal rabbit antiserum that did not cross-react with *Salmonella* group or Vi antigens. Urine specimens were tested with anti-Vi–coated and control staphylococci on the same slide. Agglutination was read after 1 min of slide agitation. A positive test was defined as agglutination with the anti-Vi–coated cells but not with the control cells. A negative test was defined as no agglutination with either anti-Vi–coated or control serum-coated cells. Any other combination was defined as uninterpretable.

For the ELISA, we used the inner 60 wells of 96-well polyvinyl microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) and ran each specimen in duplicate. Half of the wells were coated with burro anti-*S. typhi* Ty2 serum, and half were coated with normal horse serum (which cross-reacts with burro serum) as described previously (1). The absorbance of the contents of each well was read 30 min after the addition of phosphatase substrate (Sigma 104) at 410 nm with a through-the-plate photometer (Dynatech Laboratories, Inc.). The ELISA value was calculated as the ratio of the average absorbance in the Vi antigen-coated wells to the average absorbance in the control serum wells, rounded to the nearest tenth. The test was standardized by serial dilutions (100 ng to 100 pg) of a highly purified *Citrobacter* Vi antigen preparation.

Statistical methods. Discriminant analysis was performed to determine the ELISA value that maximally separated the typhoid cases from the afebrile controls. A classification function was then generated that determined the number of cases and controls that could be correctly categorized. Multiple regression analysis was used to examine the relationship between the ELISA scores of the typhoid cases and the afebrile controls controlling for age, group, hospital, prior history of typhoid, and typhoid vaccination. Linear logistic regression was performed to investigate the relationship between the coagglutination test result and case status controlling for age group, hospital, prior history of typhoid, and typhoid vaccination.

RESULTS

There were 188 culture-proven typhoid infections and 43 paratyphoid infections (Table 1). *S. typhi* was isolated from blood in 81%, from stool alone in 16%, and from marrow alone in 3% of the cases. *S. typhi* was isolated from 42% of persons suspected of having typhoid. Of the typhoid cases, 63% occurred in males, and 72% occurred in patients aged 10 to 29 years (Table

TABLE 1. Patients with typhoid or paratyphoid and control subjects, by hospital, Santiago, Chile, January and February 1982

Type of patients	Culture-proven patients		Controls	
	Typhoid	Paratyphoid	Febrile	Afebrile
Pediatric				
Inpatient	28	3	4	41
Outpatient	12	7	0	22
Adult inpatient	148	33	29	98

TABLE 2. Sex and age distribution of patients and control subjects Santiago, Chile, January and February 1982

Patients	Culture-proven patients (%)		Controls (%)	
	Typhoid (N = 188)	Paratyphoid (N = 43)	Febrile (N = 33)	Afebrile (N = 161)
Males	63	67	60	55
Age				
0–4	3	2	6	2
5–9	6	12	9	18
10–19	38	39	34	24
20–29	34	41	19	5
30–39	12	2	13	12
40–49	7	2	7	11
50–59	1	0	9	10
≥60	0	0	3	18
Unknown	8	2	12	1
Mean age	21.6	18.7	25.4	32.7

2). The mean age of afebrile control patients was significantly higher than the mean age of patients with typhoid ($P < 0.001$, Student t test) or paratyphoid fever ($P < 0.001$, Student t test).

S. paratyphi B was isolated from 32 of the 43 patients with paratyphoid fever. *S. paratyphi* B was isolated from the blood of 20 (63%) patients and from stool alone of 12 (37%) patients. *S. paratyphi* A accounted for the remaining 11 cases. In 10 patients, the organism was isolated from the blood, and in 1 it was isolated from the stool. Of the paratyphoid cases, 67% occurred in males, and 80% occurred in persons 10 to 29 years old (Table 2).

In Chile, a 100 pg/ml preparation of *Citrobacter* Vi antigen gave a mean value of 2.0 in the ELISA, 1 ng/ml gave a mean value of 6.7, and 10 ng/ml gave a near-maximum mean value of 8.3. Patients had significantly higher ELISA scores than did afebrile controls ($P < 0.0001$, Student t test; Table 3). The discriminant analysis with ELISA scores used to predict typhoid cases and afebrile controls yielded an F statistic that was significant [$F(1, 298) = 49.8$, $P < 0.0001$]. In the classification function, a case yielding an ELISA score of 1.9 or below (rounded to the nearest tenth) was classified as a noncase and one with a score of 2.0 or greater was classified as a case. This function correctly classified 62.4% of the cases and 86.8% of the controls. The effect of changing the cut-off point for a positive ELISA value on the true- and false-positivity rate is shown in Fig. 1.

Multiple regression analysis investigating the relationship between ELISA and case status, age, hospital, prior history of typhoid fever, and typhoid vaccination yielded a significant F sta-

TABLE 3. Comparison of three diagnostic tests for acute typhoid Santiago, Chile, January and February 1982

Parameter	Data for cases:			
	Culture-proven cases		Controls	
	Typhoid	Paratyphoid	Febrile	Afebrile
ELISA				
No. of cases	141	34	21	159
Mean value ± SD	2.34 ± 1.05	2.09 ± 0.47	2.08 ± 0.52	1.65 ± 0.62
95% range	1.50–4.37	1.28–2.90	1.50–3.35	1.20–2.40
Value >1.9[a]	62.4%	64.7%	47.6%	13.2%
Coagglutination				
No. of cases	145	33	24	158
% Positive	34	48	46	14
% Negative	45	33	33	46
% Uninterpretable	21	18	21	41
Widal reciprocal Ab titer				
No. of cases	174	43	4	0
O ≥ 40 (%)	78	52	25	NT[b]
O ≥ 80 (%)	72	33	0	NT
O ≥ 160 (%)	35	12	0	NT
H ≥ 40 (%)	78	55	25	NT
H ≥ 80 (%)	72	33	0	NT
H ≥ 160 (%)	35	9	0	NT
O ≥ 40 or H ≥ 80 (%)	77	44	25	NT
O ≥ 160 or H ≥ 160 (%)	41	12	0	NT

[a] Values rounded to nearest tenth.
[b] NT, Not tested.

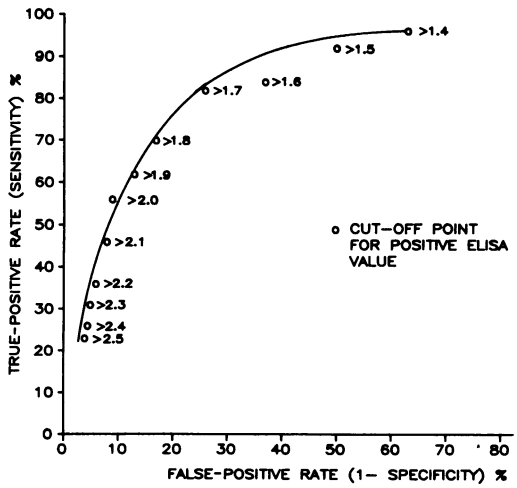

FIG. 1. Urinary Vi detection by ELISA at various cut-off points, Santiago, Chile, January and February 1982.

tistic [$F(5, 164) = 5.28$, $P < 0.01$]. Controlling for the above variables, cases had significantly higher ELISA scores than did controls [$F(1, 168) = 7.99$, $P < 0.01$].

With the slide coagglutination test, we detected 100 ng of the *Citrobacter* Vi antigen standard. The slide coagglutination test was positive in 34% of typhoid patients, 48% of paratyphoid patients, 16% of febrile control subjects, and 14% of afebrile control patients, but frequently the test result was uninterpretable, largely because of agglutination in control cells (Table 3). Typhoid cases were more likely than controls to have a positive coagglutination test when controlling for previous history of typhoid, hospital, and age ($P < 0.05$, linear logistic regression). A weak correlation between the ELISA and coagglutination test scores of typhoid cases and afebrile controls was found ($r = 0.23$, $P < 0.01$).

Some 77% of typhoid cases and 44% of paratyphoid cases had an O antibody titer of ≥1:40 or an H antibody titer of ≥1:80. Titers were not obtained from afebrile controls. Antibodies to Vi antigen were detected in the convalescent-phase serum samples of 11 of 24 patients (46%) with culture-confirmed typhoid fever. The mean Vi antibody titer was 1:80 (range, 1:20 to 1:320). In all 24 serum samples tested, H and O antibody titers were elevated. *S. typhi* isolated from patients in Chile all agglutinated in Vi-specific antisera, and *S. paratyphi* A and B isolates did not.

DISCUSSION

Based on their ability to detect purified *Citrobacter* Vi antigen, we found that the coagglutination test and the ELISA had the same sensitivity in Chile as in our laboratory in the United States. However, in Chile detection of Vi antigen in urine by either test was not a useful way to diagnose acute typhoid fever because of low sensitivity and specificity and a large number of uninterpretable test results. The ELISA, which was 100 times more sensitive than the coagglutination test in detecting the Vi antigen standard, was moderately useful in distinguishing typhoid cases from afebrile controls (specificity, 87%; sensitivity, 62%) but was not able to distinguish typhoid cases from cases of paratyphoid or from febrile controls. The high rate of false-positive ELISA and coagglutination results in febrile control subjects was particularly important since the practical application of these tests would be to help determine whether persons with febrile illnesses had typhoid fever.

The high percentage of paratyphoid illnesses with positive ELISA values was unexpected and unexplained since *S. paratyphi* A and B do not possess Vi antigen. These results suggest that our two Vi antibody reagents (burro anti-*S. typhi* Ty2 Vi and rabbit anti-*Citrobacter freundii* 5396/38 Vi) may contain antibodies which cross-react with paratyphoid antigens. Such cross-reactions would be easiest to explain for the burro antiserum which was used to coat the wells for the ELISA, since the burro antiserum was prepared against the *S. typhi* Ty2 strain and may well include antibodies to O, H, and common enterobacterial antigens as well as to Vi antigen. Theoretically, to yield a false-positive ELISA, the rabbit antiserum would also have to have antibodies to antigens which reacted with the burro antiserum or to other antigens closely linked to those antigens. *S. typhi*, *S. paratyphi* A, and *S. paratyphi* B share the O-12 antigen, but *C. freundii* 5396/38 does not, and the rabbit *Citrobacter* Vi antiserum does not agglutinate *S. paratyphi* A or B organisms. However, the ELISA and the coagglutination test are considerably more sensitive than the slide agglutination test and might detect cross-reactions that are not detectable in the agglutination of whole organisms.

Both tests were difficult to interpret because of positive reactions in normal serum-coated cells and wells, which frequently occurred in the presence of highly positive samples. This and positive reactions in febrile controls suggest that something in the urine of febrile patients is cross-reacting in these test systems. Rockhill et al. noted a similar phenomenon and suggested that urinary leucocytes could cause nonspecific agglutination which could be eliminated by centrifugation of the urine specimens before testing (7). We were unable to improve our results by this procedure.

The low sensitivity of the two tests for Vi

antigen in urine might be explained by qualitative or quantitative differences in the Vi antigen produced by the typhoid strains in Chile. Also, *Citrobacter* Vi antigens may not be immunologically identical to Vi antigen(s) from *S. typhi* (3, 9). Finally, Vi antigen may be changed physically or chemically before it appears in the urine.

Although tests of urine by the slide coagglutination test and the ELISA were not specific or sensitive in identifying persons with typhoid fever, we believe that attempts to develop and improve these and similar tools for the rapid diagnosis of the disease still offer promise and should not be abandoned. The relative lack of specificity of the reagents we used and our incomplete understanding of Vi antigens suggest that there is a need for more specific immunopurified or possibly monoclonal antibodies directed to *S. typhi* Vi in these procedures.

ACKNOWLEDGMENTS

We are grateful to the clinicians at Roberto del Rio Hospital and the Hospital de Enfermedades Infecciosas, and to Josefa Astorga, Department of Clinical Microbiology, Public Health Institute, Santiago, Chile for their help.

This work was supported in part by U.S. Army Research and Development contract DAMD 17-81-1115.

LITERATURE CITED

1. **Barrett, T. J., J. D. Snyder, P. A. Blake, and J. C. Feeley.** 1982. Enzyme-linked immunosorbent assay for detection of *Salmonella typhi* Vi antigen in urine from typhoid patients. J. Clin. Microbiol. **15**:235–237.
2. **Ewing, W. H, and W. J. Martin.** 1974. *Enterobacteriaceae*, p. 189–221. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
3. **Levin, D. M., K.-H. Wong, H. Y. Reynolds, A. Sutton, and R. S. Northrup.** 1975. Vi antigen from *Salmonella typhosa* and immunity against typhoid fever. II. Safety and antigenicity in humans. Infect. Immun. **12**:1290–1294.
4. **Levine, M. M., O. Grados, R. H. Gilman, W. E. Woodward, R. Solis-Plaza, and W. Waldman.** 1978. Diagnostic value of the Widal test in areas endemic for typhoid fever. Am. J. Trop. Med. Hyg. **27**:795–800.
5. **Nolan, C. M., J. C. Feeley, P. C. White, Jr., E. A. Hambie, S. L. Brown, and K.-H. Wong.** 1980. Evaluation of a new assay for Vi antibody in chronic carriers of *Salmonella typhi*. J. Clin. Microbiol. **12**:22–26.
6. **Ristori, C.** 1981. Epidemiología de la fiebre tifoidea en Chile. Bol. Vigil. Epidemiol. Minist. Salud Chile. **8**:8–11.
7. **Rockhill, R. C., L. W. Rumans, M. Lesmana, and D. T. Dennis.** 1980. Detection of *Salmonella typhi* D, Vi, and d antigens, by slide coagglutination, in urine from patients with typhoid fever. J. Clin. Microbiol. **11**:213–216.
8. **Schroeder, S. A.** 1968. Interpretation of serologic tests for typhoid fever. J. Am. Med. Assoc. **206**:839–840.
9. **Szewczyk, B., and A. Taylor.** 1980. Immunochemical properties of Vi antigen from *Salmonella typhi* Ty2: presence of two antigenic determinants. Infect. Immun. **29**:539–544.