JOURNAL OF CLINICAL MICROBIOLOGY, May 1977, p. 510-513

Vol. 5, No. 5
Printed in U.S.A.

Further Evaluation of the Test for Detection of Antibody-Coated Bacteria in Urine Sediment

STEPHEN R. JONES* AND JEAN JOHNSON

Veterans Administration Hospital and the University of Oregon Health Sciences Center, Portland, Oregon 97201

Received for publication 9 September 1976

To assess the reproducibility of the test for detection of antibody-coated bacteria in urine sediment as it might be used in a diagnostic laboratory for classification of urinary tract infections, multiple urine specimens from 83 patients were tested. The results were reproducible and consistent, or if inconsistent potentially explainable, in all but four patients. The explanation for inconsistencies include the immune response to the infecting bacteria, nonspecificity of the antibody coating the bacteria, antibody in prostatic secretions, and antibody-coated bacteria contaminating the urine specimens.

Proper management of urinary tract infections depends upon distinguishing bladder from upper tract infection. Recently a simple, noninvasive technique, the detection of antibody-coated bacteria (ACB) in the urine sediment, has been shown to correlate closely with upper tract infection (2, 6). The initial validation of this test was made in a relatively small number of closely studied patients (2). It is the purpose of the communication to report the results of our experience with the ACB test as it might be used in a diagnostic microbiology laboratory. The principle information sought was whether the ACB test yields consistent, reproducible results when testing multiple samples that were collected at different times from one patient. It was not the purpose of this study to assess further the reliability of the ACB test as a means of localizing urinary tract infections.

MATERIALS AND METHODS

Patients. All patients studied were middle aged men receiving care at the Veterans Administration Hospital, Dallas, Tex. They consisted of 83 in-patients with significant bacteriuria, i.e., with urine cultures containing $>10^5$ of a gram-negative bacillus (*Enterobacteriaceae* or *Pseudomonas* species) per ml, whether in pure or mixed culture (two gram-negative bacilli). To lessen the possibility that analysis was being made of contaminated urine specimens, which is a persistent problem in many clinical microbiology laboratories, a patient was deleted from the study if more than three bacterial species were recovered from a urine culture.

The patients were chosen simply by obtaining each urine sample with significant bacteriuria submitted to the clinical microbiology laboratory. These 83 patients were selected from a total of 165 successional patients because one or more follow-up urine specimens were available before initiation of therapy.

Specimens. The initial clean catch midstream urine specimen was obtained in the routine manner by the ward or clinic personnel and was transported to the clinical microbiology laboratory by hospital volunteers. The follow-up specimens were collected by the research nurse and promptly cultured. Urine from catheterized patients was obtained by aseptic needle aspiration.

Laboratory. ACB were detected in the urine by the method of Thomas et al. (6) by treating washed urine sediment with 0.25 ml of a 1:5 dilution of fluorescein-conjugated horse anti-human globulin (Roboz Surgical Instrument Co., Washington, D.C.). Negative controls were included by similarly treating a suspension of the bacterial cells recovered from culture of the same urine (2, 6). A specimen was designated as ACB(+) if at least two uniformly fluorescent bacteria were seen in a total of 200 microscopic sight fields (×195 objective and a ×15 ocular). The intensity of fluorescence was not taken into consideration. Before a specimen was designated ACB(-), the sediment was examined for 4 min, i.e., approximately 200 microscopic fields. All tests were performed by one observer who was unaware of the specimen's source. The antibody content of prostatic fluid was assayed by an indirect fluorescent-antibody technique as described by Thomas et al. (6).

RESULTS

The number of ACB in urine sediment specimens varied considerably from patient to patient and from specimen to specimen from the same patient. As mentioned above, whether there were two ACB in 200 microscopic sight fields or 200 ACB in one field, the interpretation was the same.

The results from the 83 patients with bacteriuria are listed on Table 1. They are divided into

TABLE 1. *Detection of antibody-coated bacteria in urine from 83 unselected men*

Group[a]	ACB results	No. of patients	No. of urine cultures and ACB determinations
1			
a	Multiple (+)	37	98
b	Multiple (−)	6	13
c	Multiple (−) → (+)	10	34
d	Multiple (+) → (−)	4	17
2			
a	Multiple (+)	10	36
b	Multiple (−)	2	4
3			
a	Single (+)	4	4
b	Single (−)	10	10

[a] Group 1: Multiple cultures with $\geq 10^5$ bacteria/ml, same bacteria. Group 2: Multiple cultures with $\geq 10^5$ bacteria/ml, different bacteria. Group 3: Single culture with $\geq 10^5$ bacteria/ml, subsequent culture (−) without therapy.

three groups on the basis of the urine culture results and further divided by the results of the ACB tests. Group 1 is those patients whose initial and follow-up urine cultures were positive for the same bacteria. The majority (43 of 57) of patients in this group had consistent ACB results: 37 persistently ACB(+) and 6 persistently ACB(-). The patients who were persistently ACB(+) greatly outnumbered those who were ACB(-). Ten patients converted from ACB(-) to ACB(+); four converted from ACB(+) to ACB(-). Indwelling urethral catheters were present in some patients of this group: nine patients in subgroup 1a, four in 1b, three in 1c, and two in 1d.

Group 2 consisted of patients whose cultures grew different species of bacteria on follow-up cultures. Ten were ACB(+), six of whom had effective treatment for an ACB(+) infection caused by one bacteria and who became reinfected within a short period and again were ACB(+). The other four patients had indwelling urethral catheters, and despite no attempt to treat the infection, their cultures contained different organisms on consecutive cultures. Two others had similar culture results, but the urine sediment contained no ACB. Neither had an indwelling urethral catheter.

In the third group, a single culture was positive, with subsequent cultures revealing no growth even though the patients had not been treated. Four of the 14 in this group were ACB(+). None of the ACB(+) patients and two of the ACB(-) patients had indwelling urethral catheters.

The majority of patients gave consistent results. Subgroups 1a, 1b, 2a, and 2b accounted for 55 of the 83 patients studied, and the results were reproducible. Furthermore, the results of the 10 patients in subgroup 1c were predictable on the basis of an immune response to the infecting bacteria. However, results with subgroup 2a implied nonspecificity of the bacteria-coating antibody: within a short time period a variety of species from the same patient were antibody coated. More disturbing were the few patients in subgroup 1d in whose urine ACB were present and then disappeared; this inconsistency suggested methodological problems. The urine of patients in subgroup 3 may have been contaminated during collection, but the urines of the four patients in 3a contained ACB. This suggested that bacterial contaminants can be antibody coated.

Further studies to explain inconsistent results. These results suggest that the ACB detection test can give inconsistent results under poorly controlled, but clinically quite real, conditions. The dissimilar results between this group of patients and other patients we have studied led to the formulation of explanations for the inconsistent results seen in the large unselected group.

(i) Is the urethral flora ACB(+), and if it contaminated poorly collected urine specimens, could it cause an ACB(+) determination? To answer this question, urethal exudate specimens were collected from six men with urethral catheters or poor hygiene. Each of these specimens contained numerous ACB.

(ii) Another source of variation in the test results could be the production of ACB by prostatic secretions. Previously reported results from a patient with bacterial prostatitis had established that ACB can appear in the urine sediment despite the absence of renal infection (1). In this patient, ACB were persistently present in large numbers in the urine sediment even though a direct localization procedure showed no evidence of upper tract infection. The large number of ACB seen in his urine sediment was inconsistent with these bacteria originating directly from the prostate, since bacteria are present only in small numbers in prostatic secretions in chronic prostatitis (5). Therefore, it seemed possible that antibody-rich prostatic fluid coated the bacteria while in the bladder. To determine whether prostatic fluid contained sufficient antibody activity to do this, prostatic fluid from four patients was assayed by an indirect fluorescent-antibody method (Table 2). In three of the four fluids, the titer against the bacteria isolated from the patients' urine and prostatic fluid was greater than or equal to the antibody activity against the bacteria isolated from other patients. Pa-

TABLE 2. *Cross-reacting antibody activity in prostatic fluid from patients with bacterial prostatitis*

Bacteria	Patient no.	Antibody activity of prostatic fluid from patient no.:[a]			
		1	2	3	4
Escherichia coli	1	[1,024][b]	<64	64	<64
Klebsiella pneumoniae	2	128	[512]	256	<64
E. coli	3	1024	128	[128]	<64
E. coli	4	512	512	256	[<64]

[a] Reciprocal titers; indirect fluorescent-antibody technique.
[b] Brackets indicate antibody titer of prostatic fluid from which the bacteria were cultured.

tient no. 4's fluid, however, contained no measurable antibody activity against any of these four species. This patient had greater variation in the number of ACB in his urine sediment than other patients. The relatively high titer of antibody activity against nonhomologous bacteria is consistent with the nonspecificity implicit in the results of subgroup 2a.

DISCUSSION

The need to localize urinary tract infections, i.e., to distinguish bladder from parenchymal infections, is recognized (2). The ACB detection test was developed by Thomas and co-workers (6) as a simple, noninvasive method to do this. Only the study by Jones et al. (2) has compared this method with a more direct technique of localization, and although good correlation was found between upper tract infection and the presence of ACB, additional studies must be reported before the test is accepted. The current study was designed to assess the test as it might be used in a diagnostic microbiology laboratory. In patients with persistent bacteriuria (group 1) with the same bacteria, the test gave reproducible results in 43 of 57 cases, and in 10 additional patients the test changed from ACB(-) to ACB(+), which is consistent with an immune response to the antigens of the infecting organism as the site of infection shifted from bladder to kidney and/or prostate. This shift from ACB(-) to ACB(+) has been shown to occur by day 11 in *Escherichia coli* pyelonephritis in rabbits (4). In four other patients, the test result changed from ACB(+) to (-), which implies inconsistency. Accordingly, it can be seen that in patients from whom multiple urine cultures yield the same bacterial species, if the urine sediment contains ACB (groups 1a and 1d), then there is a 90% chance (37 of 41) that the next sediment examined will also reveal ACB.

Of the 12 patients whose urine cultures grew different bacteria when cultured at various times, 10 had urine sediment in which ACB were found consistently. Six of these patients were treated and became infected. The interval between treatment and the next ACB(+) urine ranged from 3 days to 8 weeks. It seems odd that these patients with a pattern of recurrent reinfection should have ACB in their urine sediment shortly after therapy. The presence of ACB in these cases implies both that tissue invasion has occurred, whether renal or prostatic, and that a rapid immune response has taken place. An alternative explanation is that the antibody coating the bacteria has an appreciable range of cross-reactivity with nonhomologous bacteria, and when new bacteria invade previously infected tissue, they too are coated as were their predecessors. Another explanation is that nonspecific antibody is present in prostatic fluid that may enter the bladder and coat the bacteria there. This explanation is supported by the data presented in Table 2, showing the presence of cross-reacting antibody in prostatic secretions. None of these four patients, however, had multiple bacterial species isolated with persistence of ACB in their urine sediment; i.e., they were not clinically identical to the patients of subgroup 2a. Unfortunately, from no member of this category was prostatic fluid available for antibody assay.

The 14 patients in group 3 probably had positive urine cultures because of contamination. Even though contamination is much less a problem in men than women, it is still a frequently encountered problem in hospitalized patients. The cause for the ACB in four of these patients is not precisely known, but if a large number of urethral, periurethral, or perineal bacteria that are antibody-coated were placed in a relatively small volume of urine, then both significant growth and detection of ACB could occur. This explanation is supported by finding ACB in the urethral exudate of men with indwelling catheters and men with poor hygiene.

Thus, only four patients (subgroup 1d) had inconsistency in their ACB determinations that was not potentially explainable. No laboratory method is without its pitfalls, and immunofluorescent techniques are particularly prone to error because they are, to a degree, subjective. It seems unlikely that the inconsistency in this

study was caused by the inconsistencies of the fluorescein-conjugated anti-human globulin, since some form of positive and negative control was included with each day's determinations. In addition, subjective error was lessened by having a single observer read all specimens without knowing their source. A likely explanation for inconsistency is that since ureteral urine draining from an infected kidney of a well hydrated patient may contain anywhere from 10^2 to 10^5 bacteria/ml (5), and if only the bacteria from the upper tract are antibody coated, it may be impossible to detect hundreds of them among the hundreds of thousands of uncoated bacteria from the associated bladder infection. This mechanism also explains the variation in ACB seen in various specimens from the same patient. It seems reasonable to minimize this problem by processing urine collected from a relatively hydropenic patient, i.e., an early-morning specimen, after the first void has eliminated many of the uncoated bacteria that have multiplied in the bladder overnight. Whether this would improve the sensitivity of the test has not been tested. That contaminants may appear in the urine sediment as ACB is reasonable, since the secretions of the urethral and gastrointestinal mucosa contain immunoglobulins (3). Therefore, as much care should be taken in the collection of urine for ACB determination as for culture, and the presence of ACB in urine in no way validates the collection of the urine.

LITERATURE CITED

1. **Jones, S. R.** 1974. Prostatitis as cause of antibody-coated bacteria in urine (letter). N. Engl. J. Med. 291:365.
2. **Jones, S. R., J. W. Smith, and J. P. Sanford.** 1974. Localization of urinary tract infections by detection of antibody-coated bacteria in urine sediment. N. Engl. J. Med. 290:591-593.
3. **Kearns, D. H., R. J. O'Reilly, L. Lee, and B. J. Welch.** 1973. Secretory IgA antibodies in urethral exudate of men with uncomplicated urethritis due to *Neisseria gonorrhoeae*. J. Infect. Dis. 127:99-101.
4. **Smith, J. W., S. R. Jones, and B. Kaijser.** 1977. Significance of antibody coated bacteria in urinary sediment in experimental pyelonephritis. J. Infect. Dis., in press.
5. **Stamey, T. A.** 1972. Urinary infections. The Williams & Wilkins Co., Baltimore.
6. **Thomas, V., A. Shelokov, and M. Forland.** 1974. Antibody-coated bacteria in the urine and the site of urinary tract infection. N. Engl. J. Med. 290:588-590.