Antibody coated bacteria in urine of patients with recent spinal injury

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Abstract

Twenty patients with an acute spinal injury were prospectively studied to assess the clinical importance of antibody coated bacteria (ACB) in the urine and the association among the different bacterial species with a positive antibody coated bacteria test. Clinical urinary tract infection was associated with a positive ACB test on 45% of occasions. Three hundred and ninety nine urine samples containing 541 bacterial isolates were assessed for the presence of ACB; 13% were found to be positive and 87% negative for ACB; 67% of urines contained a single bacterial isolate. *Pseudomonas aeruginosa* was most commonly associated with clinical urinary tract infection, found in 25% of episodes, followed by *Proteus mirabilis* (17.5%), *Klebsiella* sp (12.5%), and *Proteus morgani* (10%). *Providencia stuartii*, however, was most commonly associated with a positive ACB test (found in 17%). Other bacteria associated with a positive ACB test included *Klebsiella* sp (14%), *Acinetobacter* sp (12.5%), *Pseudomonas aeruginosa* (12%), *Citrobacter* sp (11.5%).

A positive ACB test is not to be expected from a patient with spinal injury who has a catheter in place, and the test may provide a useful guide to identify those patients with an invasive infection. It is doubtful that a decision to treat or not treat bacteriuria could rest on the identification of the bacterial species alone.

The diagnosis and treatment of invasive urinary tract infection in patients with spinal injury and neurogenic bladder is notoriously difficult. Patients commonly have bacteriuria, but the clinical importance of this is difficult to assess as the usual clinical signs of cystitis or pyelonephritis may be absent. The antibody coated bacteria (ACB) test was developed by Thomas *et al* in 1974¹ as a means of localising the site of urinary tract infection using a non-invasive method. A positive test was taken as indicating renal infection, but it later became evident that false positive results occurred with other invasive urinary infections, such as prostatitis.

Thomas and Forland later reviewed the ACB test and concluded that the major clinical usefulness of a positive test was in distin-

guishing a simple lower urinary tract infection from an invasive urinary tract infection which would require a prolonged course of antibiotics.² The test has been used in patients with spinal injury with variable results. In 1980 Newman *et al* first described the use of the ACB test in patients with spinal injury—urine samples were obtained at the time of their annual review.³ They suggested that certain bacterial species were more likely than others to be antibody coated in the urine and would therefore warrant treatment with antibiotics. Conversely, it was suggested that infection with bacteria which were infrequently associated with a positive ACB test would be unlikely to warrant treatment in the absence of other signs or symptoms. We prospectively studied serial ACB tests in 20 patients with a recent spinal injury to assess the clinical importance of the ACB test and the association of the different bacterial species with the presence of ACB in the urine.

Methods

The patients comprised 17 males and three females, aged 12–67 years, admitted to the Regional Spinal Injuries Centre 0–38 days after injury (average seven days). Ten were tetraplegic and 10 were paraplegic. All required catheter drainage for at least 54 days and were studied for 14 days to 10 months (average six months). Daily clinical records were kept of any fever, signs or symptoms of sepsis, especially urinary tract infection, and antibiotic treatment.

Urine specimens and serum for C-reactive protein estimation were taken twice weekly, and when clinical urinary tract infection was suspected—for example, if the patient had rigors, a fever of more than 38°C for more than two hours, or positive blood cultures with significant bacteriuria ($>10^5$ organisms/l of a catheter urine or $>10^8$ organisms/l in a mid-stream sample) and pyuria (>10 white cells $\times 10^6$ /l). Specimens were cultured aerobically overnight on cysteine lactose electrolyte deficient (CLED) agar. Those yielding significant bacteriuria were stored at 4°C for investigation of ACB in the urine. Cultures considered to be contaminated—that is two, or more bacterial species isolated from a mid-stream urine on a single occasion were excluded from the analysis.

ACB TEST

A modified immunofluorescent slide method developed by Matuscak *et al* was used.⁴ Urine

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Table 1 Bacterial species associated with positive (+) and negative (–) ACB test organism isolated in pure culture

Bacteria	ACB positive (%)	ACB negative (%)	Total
<i>Acinetobacter anitratus</i>	1 (12.5)	7 (87.5)	8
<i>Citrobacter</i> sp	3 (11.5)	23 (88.5)	26
<i>Enterobacter</i> sp	0 (0)	7 (100)	7
<i>Escherichia coli</i>	0 (0)	12 (100)	12
<i>Klebsiella</i> sp	3 (14)	19 (86)	22
<i>Proteus mirabilis</i>	5 (11)	42 (89)	47
<i>Proteus morganii</i>	0 (0)	1 (100)	1
<i>Providencia stuartii</i>	7 (17)	35 (83)	42
<i>Pseudomonas aeruginosa</i>	10 (12)	76 (88)	86
<i>Enterococcus faecalis</i>	1 (6)	15 (94)	16
Coagulase negative staphylococci	0 (0)	2 (100)	2
		Total	269

(0.5 ml) was centrifuged at $1500 \times g$ for 10 minutes. The supernatant was then discarded and the sediment was washed twice in phosphate buffered saline (PBS).

Smears of the sediment were prepared by placing one drop from a sterile 50 drop pipette into the well of the multispot slide and being air dried. Once dried, the smears were fixed in acetone at room temperature for 15 minutes. The slides were then stained by adding one drop of fluorescein labelled antihuman immunoglobulin (Wellcome) to each spot and incubating in a moist chamber at 37°C for 30 minutes. Slides were washed by soaking in two changes of PBS for five minutes each. Finally, they were rinsed in distilled water and air dried. Slides were examined for fluorescence using a filter Y475. A semiquantitative assessment was made of the number of ACB present and recorded as –, +, ++, or +++. Control slides were prepared by suspending the different bacterial species isolated in PBS to just visible turbidity and then washing and staining them as above. Slides were examined for the presence or absence of fluorescence to exclude autofluorescence of any bacteria.

Results

Three hundred and ninety nine urine samples with positive culture were obtained during the study period and assessed for the presence of ACB, an average of 21 samples per patient (range five to 32). Thirteen percent of samples were positive and 87% were negative for ACB. The first urine sample positive for ACB after injury (and insertion of a catheter) was detected between five and 248 days after injury (mean 74 days) in 18 patients. During the study period 13 of the 20 patients developed 33 episodes of clinical urinary tract infection. In 32 of 33 episodes of clinical urinary tract infection

serum C-reactive protein was increased. In the one episode of clinical urinary tract infection when C-reactive protein was not increased, the ACB test was positive. All patients with clinical urinary tract infection received antibiotic treatment and the ACB test became negative in all cases after successful treatment for urinary tract infection, although in one patient the test remained positive for three weeks after a course of appropriate antibiotics had been started. On 19 occasions a positive ACB test was found when clinical urinary tract infection was not suspected. On five of these occasions an increased serum C-reactive protein concentration was also noted, raising the suspicion of a subclinical urinary tract infection, but the ACB test result became negative without specific treatment as antibiotic treatment was only given to those patients with clinical urinary tract infection.

Two tetraplegic patients who were studied for 120 and 300 days, respectively, did not at any time develop a positive ACB test despite persistent bacteriuria. One of these patients did, however, have one episode of clinical urinary tract infection during the study. In the 11 patients in whom ACB were present within two months of injury 10 were clinically known or suspected of having a urinary tract infection at the time. One patient developed a weakly positive ACB test at day 20 but was not suspected of having a urinary tract infection, and the ACB test was subsequently negative without antibiotic treatment.

ACB in the urine for the first time preceded the onset of clinical pyelonephritis. Four patients showed no signs or symptoms of urinary tract infection during the study, but three of these had ACB in the urine on at least one occasion, and in one patient this was associated with an increased C-reactive protein concentration on six separate occasions. Three other patients had a mild fever of less than 38°C with a positive urine culture and no other sites of sepsis (possible urinary tract infection). All had raised C-reactive protein concentrations and two out of three had ACB in the urine.

During the study 399 positive urine samples were obtained and 541 bacteria were isolated. *Pseudomonas aeruginosa* was the most common isolate, being present in 130 samples (24%). *Providencia stuartii*, *Proteus mirabilis*, *Klebsiella* sp were also frequently isolated in 96 (18%), 71 (13%), and 69 (12.6%) of urines, respectively. These common organisms were found in several different patients: *Ps aeruginosa*, *P stuartii*, and *P mirabilis* were isolated from 14 of 20 of the patients on at least two occasions and *Klebsiella* sp were similarly found in 13 of 20 patients. *Enterococcus faecalis*, *Acinetobacter*, and staphylococcal species were occasionally isolated in 6%, 5%, and <1% of urine samples, respectively.

Of the positive urine cultures, however, 269 (67%) contained organisms in pure culture and 130 (33%) were mixed cultures. Table 1 shows the different bacterial species isolated from urine samples containing pure cultures only. Again *Ps aeruginosa* was the commonest isolate found in 86 of 269 (32%) urine samples. *P*

Table 2 Different bacterial species associated with clinical urinary tract infection

Bacterial isolate	Number isolated	Percentage of total
<i>Pseudomonas aeruginosa</i>	10	25
<i>Proteus mirabilis</i>	7	17.5
<i>Klebsiella</i> sp	5	12.5
<i>Proteus morganii</i>	4	10
None	4	10
<i>Citrobacter</i> sp	3	7.5
<i>Providencia stuartii</i>	3	7.5
<i>Enterococcus faecalis</i>	2	5
<i>Enterobacter</i> sp	1	2.5
<i>Escherichia coli</i>	1	2.5
Total	40	

mirabilis and *P. stuartii* were isolated from 17% and 16% of urines, respectively. *P. stuartii*, however, was most commonly antibody coated, the ACB test being positive in 17% of urines containing this organism in pure culture. *Klebsiella* sp, *Ps. aeruginosa*, *Citrobacter* sp and *P. mirabilis* were also common isolates which were antibody coated in 10-15% of urines from which they were isolated (14%, 12%, 11.5%, and 11%, respectively). *A. anitratus* was infrequently isolated but found to be ACB positive on one occasion. In contrast, *Enterobacter*, *E. coli*, *P. morganii* and staphylococci were infrequently isolated and not found to be antibody coated when isolated in pure culture.

Seven per cent of the mixed urine cultures were positive for ACB and yielded 46 different isolates. *Ps. aeruginosa* and *P. stuartii* were commonly associated with a positive ACB test. The clinical importance of a positive ACB test with mixed urine cultures, however, is difficult to assess.

If the different bacterial species associated with clinical urinary tract infection are assessed (table 2) the results indicate that *Ps. aeruginosa* was most commonly found (25%), followed by *P. mirabilis* (17.5%), *Klebsiella* sp (12.5%), and *P. morganii* (10%). *P. stuartii* and *Citrobacter* was found less frequently (7.5%). In six of the episodes mixed bacterial cultures were obtained, and on four occasions no bacterial growth was obtained from the urine samples.

Discussion

Clinical symptoms and signs of urinary tract infection are notoriously unreliable for determining the site of urinary tract infection especially in the patient with spinal injury who depends on a catheter. The problems of diagnosing urinary tract infection in the patient with a spinal cord injury caused us to investigate the use of non-invasive techniques as a guide to invasive urinary tract infection in these patients because antibiotics should be reserved for those with an invasive infection. The presence of ACB in the urine had been interpreted by previous workers as an indication of infection in the kidney.¹⁵ This was later questioned when it was realised that antibody could be secreted at any level of the urinary tract when tissue invasion had occurred.⁶ Our previous work indicated that C-reactive protein may be a useful guide in the management of urinary tract infection in patients with spinal injury.⁷ We have now studied the use of the ACB test in patients with a recent spinal injury with a view to assessing the clinical importance of a positive test and identifying the different bacterial species associated with a positive test in patients who have not previously required the long term use of a catheter.

Direct tests for localisation of urinary tract infection, such as ureteric catheterisation⁸ and the bladder washout test⁹ are most reliable but uncomfortable, may even be hazardous, and are too time consuming to be adopted as a routine clinical procedure on a regular basis for several patients. In 1974 Thomas and co-workers

developed the technique of detecting antibody coated bacteria in urine.¹ Since then several studies have looked into the usefulness of the test for predicting the site of infection comparing the ACB test with direct methods.¹⁰ Mundt and Polk reviewed the use of the ACB test in 1979 and found an overall sensitivity of 83.1% and a specificity of 76.7%.¹¹ The positive predictive value was 81.3% and the negative predictive value 78.8%. Non-standardisation of the ACB test, however, produced a range of results from different workers. Gargan *et al* attempted to standardise the ACB test.¹² They found that patients with urinary tract infection and low counts of ACB—that is <1%—responded as well as those with a negative ACB to treatment for urinary tract infection. The presence of more than 1% ACB in a mid-stream urine specimen identified those patients who were at a high risk of treatment failure—that is, those who had “more than a simple lower tract infection”.

The ACB test has been studied in several different groups of patients—those with simple or recurrent urinary tract infection,^{15 12} spinal injury and neurogenic bladder,^{3 13-17} and in children.¹⁸ Diverse opinions have emerged regarding the usefulness of the test in each group. Studies on patients with neurogenic bladder have produced conflicting results. Some workers indicated that the limitations of the test precluded its use.^{13 15 17} Others felt that the test could be of some benefit while acknowledging its limitations.^{3 14 16}

Newman *et al* studied 121 urine samples from patients with spinal cord injury at the time of annual review.³ Sixty-six (55%) of these had a positive ACB test (compared with 13% in our study). Of the positive culture results, 42% contained a single bacterial isolate (compared with 67% in our study). They found that *E. coli* was the commonest single isolate and antibody coated in 54% of urines. This was an uncommon isolate in our centre. In contrast, we found *P. stuartii* was a common isolate and the organism most commonly antibody coated. *Ps. aeruginosa* seemed to be a predominant organism in both centres. The discrepancy in results may have been due to the fact that patients in our study were those with a recent spinal injury, but in Newman's study the patients had more long standing injury. Despite the high percentage of positive tests Newman *et al* concluded that the ACB test is a useful diagnostic aid for this group of patients for whom more invasive methods would be inappropriate.³

Lindan studied 234 patients two months to 14 years after injury (average four years) and found that the patients' urine was positive for ACB in 34% of samples.¹⁶ They found that *Klebsiella* and *Enterobacter* were most commonly antibody coated and it was suggested that, “infection with these organisms should be treated promptly and vigorously”. The study also indicated that *Pseudomonas*, *Serratia*, *P. stuartii* and *Citrobacter* were practically never antibody coated, and it concluded that isolation of these organisms in the urine did not necessarily warrant antibiotic treatment. Our results suggest that *Pseudomonas*, *Providencia*, and

Citrobacter cannot be assumed to be harmless opportunist organisms as they were often associated with a positive ACB test and clinical urinary tract infection.

Our results indicate that although certain bacteria may be more commonly antibody coated than others, it is doubtful that a decision to treat or not to treat bacteriuria in the patient with a spinal injury could rest on the identification of infecting bacterial species alone. Clinical signs and symptoms are also unreliable in predicting which patients require treatment. Most of our patients with clinical evidence of urinary tract infection had increased C-reactive protein concentrations, but only 45% had a positive ACB test result, indicating that there may be a group of patients who have a more severe invasive infection. Conversely, the presence of a positive ACB test in the absence of any clinical symptoms or signs and a normal C-reactive protein may be interpreted as a possible false positive result—by repeating the urine culture, ACB test, and estimating serum C-reactive protein concentration, it should be possible to clarify the meaning of the test. The lower incidence of positive ACB test results in the urines of our patients than in those reported in other studies indicates that perhaps the ACB test may be more useful in the management of patients with a recent spinal injury than in those who are being assessed at annual review. A combination of clinical assessment, the ACB test, and C-reactive protein estimation may provide a simple screening procedure to distinguish those patients who have bacteriuria alone from those who have an invasive infection.

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