Comparison of a New Enzyme-Linked Immunosorbent Assay Method with Counterimmunoelectrophoresis for Detection of Teichoic Acid Antibodies in Sera from Patients with Staphylococcus aureus Infections

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Ribitol-teichoic acid antibodies were measured by a new enzyme-linked immunosorbent assay (ELISA) and by counterimmunoelectrophoresis in serum samples from 47 patients with serious Staphylococcus aureus infections, 63 infected patients, and 177 healthy controls. The same antigen was used for both tests. The group of patients with S. aureus endocarditis (6 patients) had significantly higher ELISA readings than the patients with other deep-seated infections (26 patients) or with an uncomplicated S. aureus bacteremia (15 patients). The patients with other serious gram-positive (40 patients) or gram-negative (23 patients) infections did not differ from the healthy control group. There were only three (7.5%) low-level cross-reactions among the infections caused by gram-positive organisms other than S. aureus. Of 46 initially ribitol-teichoic acid antibody-negative patients followed up for 2 weeks or more, only those developing a serious S. aureus infection showed a significant rise of the ELISA reading. There was a good correlation between ELISA and counterimmunoelectrophoresis. Both tests could be useful in the diagnosis and the management of complicated S. aureus infections. The ELISA method is, however, more sensitive and usually reflects the antibody rise after an infection earlier than does counterimmunoelectrophoresis.

Staphylococcus aureus infections are an important problem in the United Kingdom. In 1980, 18% of all community and hospital-acquired infections among inpatients were due to this organism (9), and in the period 1975 to 1980, S. aureus was responsible for 18% of all significant bacteremias reported to the Communicable Diseases Surveillance Centre (17).

Measurement of antibodies to ribitol-teichoic acid (R-TA), the major antigenic component of the cell wall of S. aureus, has shown considerable potential in the diagnosis and management of serious S. aureus infections. Thus, the detection of rising R-TA antibody titers, or particularly high titers, has proved useful in distinguishing between a "complicated" bacteremia, needing prolonged treatment, and a transient bacteremia, and in diagnosing a serious, deep-seated infection such as S. aureus endocarditis (6, 10, 15). Cross-reactions have been reported occasionally with R-TA antibody tests in patients infected with Staphylococcus epidermidis (4), various streptococci (10), and diphtheroids (8). It has been suggested that there are also cross-reactions with lactobacilli, bacilli, and Haemophilus influenzae (1).

R-TA antibodies have previously been detected by either gel diffusion or counterimmunoelectrophoresis (CIE) (4, 6, 13). Both techniques have limitations, and a better technique is badly needed (12). Recently, several enzyme-linked immunosorbent assay (ELISA) methods have been published, measuring antibodies against either staphylococcal R-TA (5, 16) or peptidoglycan (14).

This report gives details of a new ELISA for R-TA antibodies that has a number of advantages over CIE.

MATERIALS AND METHODS

Patients and controls. The groups of patients are described in Table 1. The 177 healthy controls were selected from among hospital staff, patients of pediatric and geriatric wards who were devoid of any acute illness or chronic skin condition, and blood donors. The patients usually were bled within 48 h after the bacteriological diagnosis was made. All sera were stored at −20°C before use.

Antigen preparation. The R-TA antigen was prepared as described by Sheagren et al. (13). Briefly, S. aureus H6571 was cultured for 48 h at 37°C on brain heart infusion agar; the organisms were harvested and washed in saline, killed with sodium azide, and finally disrupted (≥95% as judged by Gram stain) with Ballotini glass beads (Dyon-Mill, W. Bachofen, Basel). The crude suspension was dialyzed overnight at 4°C against polyethylene glycol (molecular weight, 6,000; BDH, Poole, England) to reduce the volume. After trypsin treatment and boiling, the suspension was pelleted by ultracentrifugation at 10,000 rpm for 30 min. The resulting supernatant was then dialyzed against distilled water for 2 days at 4°C. The final solution was microfiltered (pore size, 0.2 μm), divided into aliquots, and stored at −20°C. The immunological identity of the antigen was checked against purified α- and β-R-TA obtained from G. W. Ross (Glaxo Laboratories, Greenford, England), using CIE and a known positive serum. The same preparation of the R-TA antigen was used for both CIE and ELISA.

CIE (13). Microscope slides (76 by 25 mm) were coated with 2.5 ml of molten 1% Noble agar in barbitone-acetate buffer (0.05 M, pH 8.6) which was left to set. Two rows of...
The optimal antigen dilution, determined by chessboard titrations, was 1:2. A high-titer serum was run on each slide as a positive control. The slides were electrophoresed with barbitone-acetate buffer (0.05 M, pH 8.6) for 30 min at a constant current of 10 mA and then examined for lines of precipitation. CIE titers of ≥1:2 were regarded as positive (13).

ELISA. (i) Preparation of conjugate and substrate solutions. The conjugate was prepared by using the periodate method (11). Protein A (10 mg; Pharmacia Fine Chemicals) was conjugated to 3 mg of horseradish peroxidase type VI (Sigma Chemical Co.). This was then diluted in an equal volume of glycerol and stored at 4°C. The substrate 3,3',5,5'-tetramethylbenzidine (TMB) (Miles Laboratories) was prepared by dissolving 10.1 mg of TMB in 1 ml of dimethyl sulfoxide. Then 200 μl of this mixture was added to 20 ml of 0.1 M sodium acetate-citric acid buffer (pH 6.0) and mixed well. Before use, hydrogen peroxide (BDH) was added to a final concentration of 1.3 mmol/liter (2).

(ii) Test procedure. The R-TA antigen was diluted 1:600 in 0.1 M carbonate buffer (pH 9.6), and 200 μl was added to the wells of Nunc ELISA-grade microtiter plates which were then incubated at 37°C for 48 h; the plates were unsealed to dry the antigen onto the wells. Coated plates were stable for at least 10 days at 37°C and for 6 months at room temperature. Before use, the plates were washed three times in phosphate-buffered saline (Oxoid PBS “A” tablets) containing 0.1% Tween (Sigma) and blotted dry. Serum to be tested was diluted 1:6,400 in the saline-Tween solution (thorough mixing was essential), and 200 μl was added to duplicate wells. R-TA antibody-positive and -negative control sera were included on each plate, together with a blank containing diluent only. The plates were sealed and incubated for 2 h at room temperature in a humid container. After washing, 200 μl of a 1:40,000 dilution of the protein A-peroxidase conjugate was added to each well. After a further 2 h at room temperature, the plates were washed and 200 μl of TMB substrate was added to each well. The reaction was allowed to proceed at room temperature for 10 to 15 min and then stopped by the addition of 50 μl of 2 M sulfuric acid. The results were read at 450 nm and were then corrected so that the mean optical density (OD) value for the positive control serum equaled 1.00. Optimum dilutions of R-TA antigen, serum, and conjugate were determined by chessboard titrations. OD values of ≥0.500 were regarded as positive.

Statistics. The results from the various diagnostic groups (Table 1) were compared by using an unpaired Student t test.

RESULTS
The overall results obtained with the initial serum samples of the various diagnostic groups are shown in Fig. 1.

(i) Healthy controls. The healthy control groups (F and G) were divided into age groups ranging from 1 to 5 years up to 71 to 90 years (Fig. 2). In the ELISA, the pediatric control group (mean OD, 0.072) had a significantly lower OD reading (P < 0.001) than the adult controls (mean OD, 0.140). Adult levels of R-TA antibody are gradually reached at approximately 15 years of age. This study did not examine children under 6 months of age.

![FIG. 1. R-TA antibodies (ELISA) in S. aureus infections (A to C), infected (D and E), and healthy (F and G) controls. Diagnostic groups: (A) S. aureus endocarditis (the patient with OD 0.111 died within 48 h after onset of illness); (B) other deep-seated S. aureus infections; (C) uncomplicated S. aureus bacteremia; (D) gram-positive bacteremia-septicemia; (E) gram-negative bacteremia-septicemia; (F) adult healthy controls; (G) pediatric healthy controls. The positive ELISA cutoff was OD ≥ 0.500; the cutoff value for positive CIE was ≥1:2.](http://jcm.asm.org/)
less than 1 year old; although it has been shown that higher levels of R-TA antibody are present in the first 6 months of life due to persisting maternal R-TA antibody (5). Within the adult control group, there were no significant differences between the age groups. All ELISA and CIE results were negative.

(ii) *S. aureus* and other infections. In the ELISA, the mean OD reading for the patients with *S. aureus* endocarditis (group A) was significantly greater than the values for all other groups (P < 0.001). The mean OD value for deep-seated *S. aureus* infections (group B) was significantly higher (P < 0.001) than the values for the infected and healthy control groups (D and G), but only the eight blood culture positive cases among group B had higher OD values (P < 0.05) than the uncomplicated *S. aureus* bacteremias (group C). The values for the patients with acute (six patients) or chronic (six patients) osteomyelitis and acute (five patients) or chronic (two patients) postoperative bone infections within group B did not differ significantly from each other.

A group of five patients with chronic atopic eczema (not shown in Fig. 1) had a mean ELISA reading of 0.579 (standard deviation, 0.287); three of them had had repeated skin infections with *S. aureus*. Only two of them had a positive CIE result (1:2 and 1:4).

It was unexpected to find in two serious *S. aureus* patients, the first a patient with acute endocarditis and the second with multiple abscesses and septicemia, negative CIE and ELISA results (ELISA ODs, 0.111 and 0.049). Both patients, however, died within 48 to 72 h after the onset of their illness, and no follow-up samples could be taken.

(iii) Follow-up of *S. aureus* infections. A total of 46 patients were followed up serologically for a period of at least 2 weeks. Seven of a total of 23 initially R-TA antibody-negative patients with a deep-seated *S. aureus* infection or bacteremia (Fig. 3I) developed serious complications (endocarditis, 3; arthritis, 1; osteomyelitis, 1; abscess, 2). They all showed a significant rise in ELISA OD (P < 0.001) between day 1 and day 14. None of the other 16 patients with an initially R-TA antibody-negative *S. aureus* infection progressed further under treatment or developed a serious complication (Fig. 3II), and no significant rise in ELISA OD was seen. Likewise, none of the 23 patients with other gram-positive or gram-negative bacteremias and septicemias (Fig. 3III and IV) showed a rise in ELISA OD.

The CIE and ELISA results of six patients with serious *S. aureus* infections followed up for a longer period are shown in Fig. 4. The overall agreement between ELISA and CIE results was good; however, in patients C, D, E, and F, ELISA detected an antibody rise 7 to 10 days earlier than did CIE. Patient F maintained a high level of R-TA antibody as measured by ELISA, although not by CIE, after repeated *S. aureus* wound infections.

(iv) Cross-reactions. Only three low-level cross-reactions were observed among the 40 infections caused by gram-positive organisms other than *S. aureus*, and none were observed among the gram-negative infections: endocarditis (one patient) and septicemia (one patient) with coagulase-negative staphylococci (ELISA OD, 0.323/0.678; CIE:

FIG. 2. Age distribution of R-TA antibodies detected by ELISA in 30 pediatric (1 to 15 years) and 147 adult (16 to 91 years) healthy controls. The connecting line shows mean values; bars in each column indicate mean values ± two standard deviations.

FIG. 3. Rise of ELISA OD values after a 2-week follow-up in 46 patients with initially R-TA antibody-negative *S. aureus* and other infections. Diagnostic groups: (I) complicated (see the text) *S. aureus* bacteremia; (II) uncomplicated *S. aureus* bacteremia or nonprogressing, deep-seated *S. aureus* infection; (III) gram-positive bacteremia-septicemia; (IV) gram-negative bacteremia-septicemia.
as allowed in of protein of antibiotic inadequate postoperative infections. by the findings of other authors antibodies with addict and antibiotics with ELISA and CIE. (A) heroin addict with recurrent endocarditis; (B) acute endocarditis in a patient with bicuspid aortic valve; (C) acute septic arthritis; (D) postoperative bacteraemia developing into acute endocarditis after inadequate antibiotic treatment; (E) fatal case of spinal osteomyelitis, kidney abscess, and empyema in an elderly diabetic; (F) acute septic arthritis with skin breakdown in a patient with severe chronic polyarthritis (repeated wound infections with \( S. \) aureus). Duration of illness: acute (<1 week) in patients B, C, D, and F; 4 weeks in patient E; chronic in patient A.

1:4/negative) and septicemia (one patient) with \( \text{Streptococcus milleri} \) and \( \text{Clostridium sp.} \) (0.507; negative).

**FIG. 4.** Examples of serious \( S. \) aureus infections followed up serologically for R-TA antibodies with ELISA and CIE. (A) heroin addict with recurrent endocarditis; (B) acute endocarditis in a patient with bicuspid aortic valve; (C) acute septic arthritis; (D) postoperative bacteraemia developing into acute endocarditis after inadequate antibiotic treatment; (E) fatal case of spinal osteomyelitis, kidney abscess, and empyema in an elderly diabetic; (F) acute septic arthritis with skin breakdown in a patient with severe chronic polyarthritis (repeated wound infections with \( S. \) aureus). Duration of illness: acute (<1 week) in patients B, C, D, and F; 4 weeks in patient E; chronic in patient A.

The specificity of both tests in this study was high, as shown by the low number and low level of cross-reactions observed. Good agreement (Fig. 1) might be expected as both tests employed the same antigen preparation (13). The choice of protein A conjugate in the ELISA test was made to reduce the high background levels found when anti-human immunoglobulin G conjugates were used. TMB was chosen as the substrate in the ELISA for a number of reasons: it allowed higher dilutions of the antigen and antisera to be used in the test compared with \( \alpha \)-phenylene diamine, and this increased the positive/negative ratios; the color produced was more stable than with \( \alpha \)-phenylene diamine, and TMB is noncarcinogenic (2). Because of the high dilution of serum (1:6,400) used in the ELISA, it is of paramount importance to ensure thorough mixing of the sample and the sample dilution. The quality of every batch of plates must be carefully checked, as problems with certain batches were experienced. Coefficients of variation across individual plates should be less than 10%.

High levels of R-TA antibody on admission, of at least twice the cutoff levels, are suggestive of a serious, deep-seated \( S. \) aureus infection (patients A and E in Fig. 4). Chronic skin conditions such as atopic eczema or leg ulcers can also give rise to abnormally high R-TA antibody levels, and these conditions should be excluded before diagnosis of a deep-seated \( S. \) aureus infection. Serum samples taken at least 1 week apart from the same patient that give more than a twofold rise of the ELISA reading, with an elevation above at least two standard deviations of the mean of the corresponding pediatric or adult control group, can be regarded as a significant change in antibody level (patients B, C, and D in Fig. 4). The monitoring of serial samples after the diagnosis of an \( S. \) aureus bacteraemia can differentiate clearly within 1 to 2 weeks between a transient bacteraemia and a complicated bacteraemia that necessitates prolonged antibiotic treatment (6). C. A. Miller, I. R.-T. Miller, J. J. M. B. Miller, G. W. M. G. Miller, and R. J. K. Miller, Proc. Int. Congr. Chemother. 13th, Vienna, Austria, part 63, (1983).

Although CIE and ELISA are of similar diagnostic value, the ELISA described in this study is more sensitive and therefore detects rises in R-TA antibody earlier than does CIE, and also reflects more accurately changes in lower levels of antibody. Not only is this useful for the earlier diagnosis of deep-seated infections, but it may also be of particular use for monitoring the course of deep-seated \( S. \) aureus infections of children, in whom R-TA antibody levels are lower than those in adults.

After a deep-seated \( S. \) aureus infection, levels of R-TA antibody return to normal within 2 to 3 months, providing the organism has been eradicated successfully (patients B, C, and D in Fig. 4). A fulminating \( S. \) aureus septicemia leading to death within 48 to 72 h after the onset of the illness may not give a high level of R-TA antibodies (4), as we experienced in two cases. Probably the detection of circulating R-TA antigen (7) is of more use in such cases. Very low or normal R-TA antibody levels are also seen in longstanding cases of \( S. \) aureus osteomyelitis (5, 8). The absence of raised antibody levels in these conditions supports the view that a positive R-TA antibody result is more indicative of the duration and the degree of antigenemia than the site of the \( S. \) aureus infection (10, 15). It was therefore to be expected that only those patients in the group with deep-seated \( S. \) aureus infections who had positive blood cultures showed significantly higher ELISA readings than those with a transient \( S. \) aureus bacteraemia.

In conclusion, both CIE and ELISA are useful rapid screening tests for the presence of R-TA antibodies; however, ELISA is more sensitive and therefore, in some cases, may be more useful in the earlier diagnosis and treatment of deep-seated \( S. \) aureus infections.

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The page contains text and diagrams related to the detection of infectious agents using ELISA and CIE methods, with specific examples of serious \( S. \) aureus infections. It discusses the specificity and sensitivity of the tests, the conditions under which high levels of R-TA antibodies are observed, and the implications of these results for patient management. The text also acknowledges the support of various individuals and institutions in conducting the research. The diagrams illustrate the progression of ELISA and CIE results over time, with different patterns for chronic and acute infections.
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LITERATURE CITED