

Serological Diagnosis of *Staphylococcus aureus* Osteomyelitis

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We have evaluated serological tests for the diagnosis of *Staphylococcus aureus* osteomyelitis. Antiteichoic acid antibodies were elevated in 17 of 23 patients with acute and 16 of 46 with chronic *S. aureus* osteomyelitis but in none of 33 patients infected with other gram-positive or gram-negative bacteria. Immunoglobulin G antibodies to *S. aureus* were elevated in 12 of 23 patients with acute and 22 of 47 with chronic *S. aureus* osteomyelitis, in 2 of 12 infected with other gram-positive bacteria, and in 4 of 21 with other gram-negative bacteria. Assays for *S. aureus* antibodies may be useful for identifying patients with *S. aureus* bacteremia complicated by metastatic sites of infection in bone and for identifying the etiological agents in patients with negative or mixed cultures or from whom cultures are not readily available. Prospective studies are needed to test these hypotheses.

Identification of the etiological agent in osteomyelitis may be difficult. In some patients with acute osteomyelitis blood cultures may be negative, thus necessitating bone biopsy to establish a diagnosis (3, 13). Furthermore, cultures of open wounds or draining sinus tracts in chronic osteomyelitis are often unreliable (14). Organisms isolated from these specimens may only represent colonization of the superficial site (5, 8), and less frequently the causative agent may not be identified in cultures from the sinus or wound (5). Also, unsuspected metastatic bone involvement may complicate nosocomial *Staphylococcus aureus* bacteremia secondary to infected intravascular foreign bodies. A prompt etiological diagnosis and appropriate therapy decrease the incidence of chronic infection in patients with acute *S. aureus* osteomyelitis (1, 4). Appropriate antibiotic therapy also requires accurate identification of the causative agent of chronic osteomyelitis.

Serological tests for antibodies to several different *S. aureus* antigens have been used in diagnosing serious staphylococcal infections (2, 6, 7, 11, 12, 15-17). These tests may provide a specific etiological diagnosis before blood cultures are identified to be positive in bacteremic infections (2, 6). Elevated levels of staphylococcal antibodies beyond the normal range also distinguish *S. aureus* bacteremia complicated by endocarditis or metastatic infection from uncomplicated bacteremia associated with infected intravascular devices, thus providing useful information in determining the duration of antibiotic therapy (7, 12, 15-17). Detection of elevated levels of anti-staphylococcal antibodies supports a presumptive diagnosis of *S. aureus* infection in patients with negative cultures because of prior antibiotic therapy.

Serological tests for staphylococcal antibodies should be useful in osteomyelitis for more rapid determination of the etiology than culture in acute infections. In this study we report our experience with the serological diagnosis of *S. aureus* osteomyelitis.

MATERIALS AND METHODS

Patients. (i) Acute *S. aureus* osteomyelitis. Each of these 23 patients had complaints of less than 6 weeks in duration and roentgenographic or radionuclide evidence, or both, for os-

teomyelitis. *S. aureus* was cultured from the blood in 18 of these patients and from bone in the remaining 5. A paraspinous abscess and a pneumonia complicated the osteomyelitis in one patient each.

(ii) Chronic *S. aureus* osteomyelitis. These 47 patients had bone infections of at least 6 weeks in duration, roentgenographic evidence of osteomyelitis, and cultures of bone containing *S. aureus* alone (43 patients) or mixed with other bacteria (4 patients). The other organisms isolated from the four with mixed cultures were *Morganella morganii* and alpha-streptococci in one each, *Serratia marcescens* and *Pseudomonas aeruginosa* in another, and *Peptococcus prevotii*, *S. epidermidis*, and alpha-streptococci in the fourth. None had *S. aureus* bacteremia.

(iii) Other gram-positive bacteria. These 12 patients had chronic osteomyelitis caused by *S. epidermidis* in 5, streptococci in 4, *Corynebacterium* sp. in 2, and *Propionibacterium* sp. in 1.

(iv) Gram-negative bacillary osteomyelitis. These 21 patients had chronic osteomyelitis caused by *P. aeruginosa* in 11, *Proteus mirabilis* in 3, *Bacteroides fragilis* and *Escherichia coli* in 2 patients each, and *M. morganii*, *Pasteurella multocida*, *Enterobacter cloacae*, and *Serratia marcescens* in 1 patient each.

Serological methods. (i) Teichoic acid antibody assay. The methods of preparing ultrasonic staphylococcal antigens and detection of antibody by agar gel diffusion have been described previously (2, 15). Sera were titrated against an undiluted ultrasonic extract of the Lafferty strain of *S. aureus*. The highest positive serum dilution was reported. In this assay teichoic acid precipitins in undiluted serum were considered positive.

(ii) Solid-phase radioimmunoassay. Solid-phase radioimmunoassay has been described previously (16, 17). Briefly, polystyrene tubes were coated with 0.2 ml of an ultrasonic extract of the Wood 46 strain of *S. aureus* diluted in 0.01 M Tris-hydrochloride buffer (pH 7.0) for 1 h at 37°C. Next, 0.2 ml of 5% bovine serum albumin in the 0.01 M Tris-hydrochloride buffer was incubated for 1 h at 37°C. Then 0.2 ml of sera diluted in 0.10 M Tris-saline (pH 8.0) with 5% bovine serum albumin (1/300 for the immunoglobulin M [IgM] assay and 1/3,000 for the IgG assay) was incubated in the antigen-coated tubes for 1 h at 37°C. Finally, 0.2 ml of ¹²⁵I-labeled goat IgG anti-human IgG at a concentration of about 2 µg/ml

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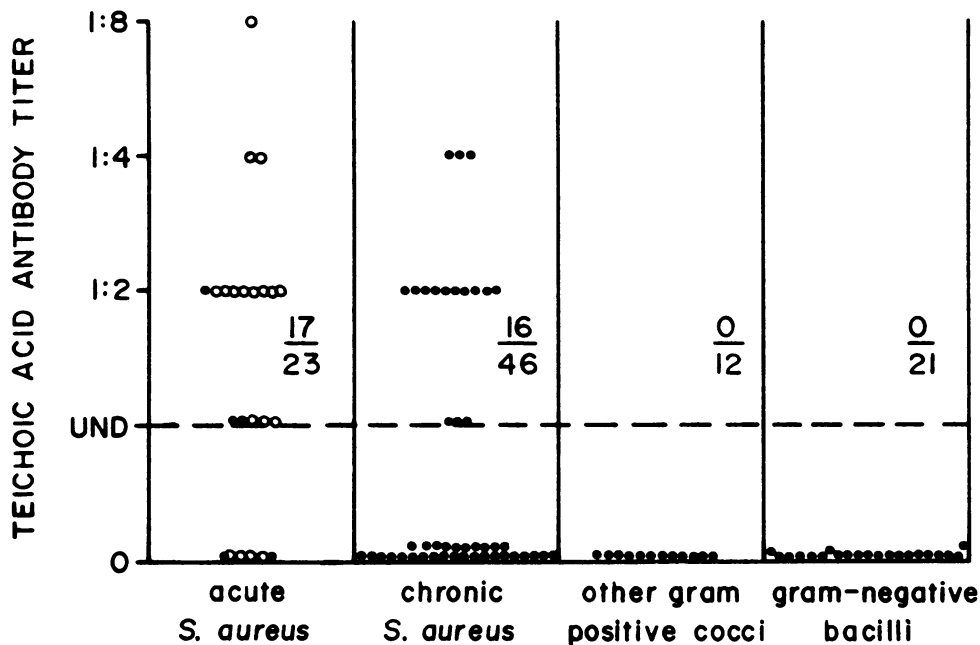


FIG. 1. Teichoic acid antibodies measured by immunodiffusion in patients with osteomyelitis. 0 = Patients with *S. aureus* bacteremia. Undiluted (UND) or diluted sera forming a precipitin line against ribitol teichoic acid were regarded as positive, shown above the broken horizontal line. The numbers within the bar refer to the number of patients with elevated antibody levels over the number tested.

in 0.10 M Tris-saline with 5% bovine serum albumin was incubated for 1 h at 37°C. Tubes were aspirated, washed three times between each step and five times after the last step, and then counted in a gamma counter. Each specimen was tested in triplicate, and results were compared to a

group of 10 sera from healthy normal adults to determine positivity. A positive control serum from a patient with *S. aureus* endocarditis was also included with each assay. The cutoff distinguishing positive and negative results drawn at 2.2 times the mean of the 10 normal sera was normalized to

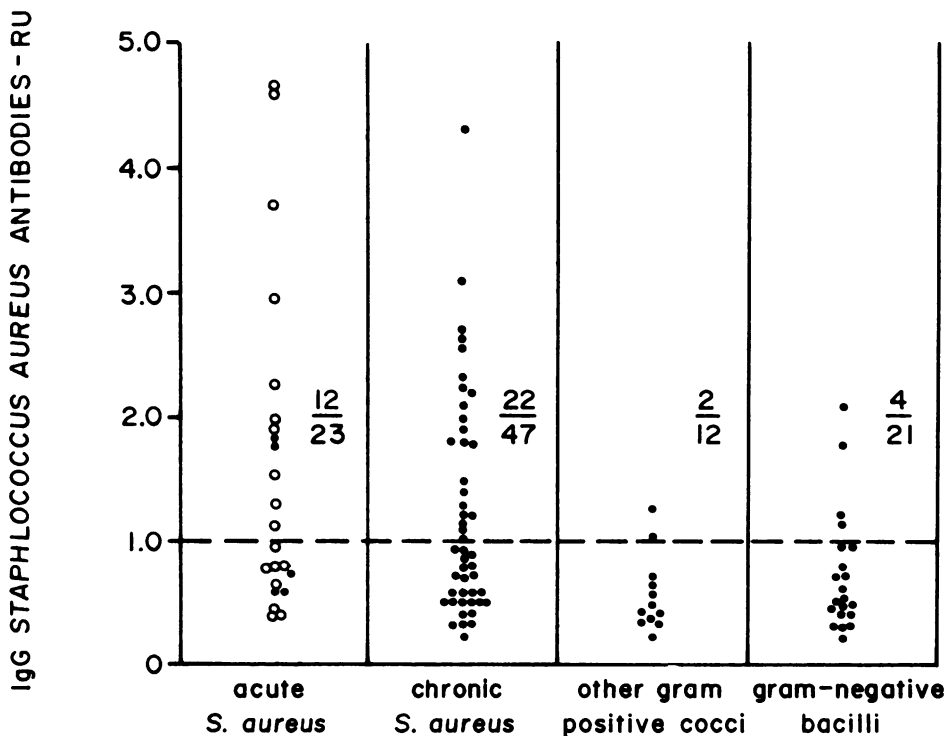


FIG. 2. IgG *S. aureus* antibodies measured by solid-phase radioimmunoassay in patients with osteomyelitis. The broken horizontal line separates positive from negative results. RU, Radioimmunoassay units (see text).

represent 1.0 radioimmunoassay unit, and all test results were multiplied by the same factor. This normalization procedure was developed to facilitate comparison of results of different experiments and is justified by the excellent interassay reproducibility of this radioimmunoassay (17).

RESULTS

Titers of teichoic acid antibodies were elevated in 17 of 23 (73.9%) patients with acute compared with 16 to 46 (34.8%) with chronic osteomyelitis caused by *S. aureus* (Fig. 1) ($P = 0.002$; Fisher's exact test). Teichoic acid antibodies were not detected in 33 individuals with osteomyelitis caused by other gram-positive or gram-negative organisms. Teichoic acid antibody titers in positive sera were similar for both acute and chronic *S. aureus* osteomyelitis.

IgG antibodies to *S. aureus* were increased in 12 of 23 (52.2%) patients with acute and 22 of 47 (46.8%) with chronic osteomyelitis caused by *S. aureus* (Fig. 2) ($P = 0.433$). Mean levels of IgG antibodies in sero-positive patients with chronic *S. aureus* osteomyelitis were similar to those in patients with acute osteomyelitis. IgM and IgG antibodies were increased with almost equal frequency in acute *S. aureus* osteomyelitis: 10 of 23 (43.5%) versus 12 of 23 (52.2%), $P = 0.384$. However, IgG antibodies were elevated more frequently in chronic osteomyelitis than were IgM antibodies: 22 of 47 (46.8%) versus 6 of 47 (12.8%), $P < 0.001$. IgG antibodies to *S. aureus* were also elevated slightly more frequently than were teichoic acid antibodies in chronic *S. aureus* osteomyelitis: 22 of 47 (46.8%) versus 16 of 46 (34.8%), $P = 0.166$. IgG antibodies were increased in 2 of 12 (16.5%) controls with osteomyelitis caused by other gram-positive bacteria, *Enterococcus* sp. in one and *Propionibacterium acnes* in another. IgG antibodies were increased in 4 of 21 (19.0%) patients with gram-negative osteomyelitis; two of these patients were intravenous drug addicts with *P. aeruginosa* osteomyelitis, one was diabetic with *M. morgani* osteomyelitis related to chronic foot ulcers, and the final patient had *Proteus mirabilis* osteomyelitis.

DISCUSSION

S. aureus is the most common cause of osteomyelitis, isolated in about 60% of cases of acute and chronic osteomyelitis (3, 5, 13). Successful antibiotic therapy requires a prompt and accurate diagnosis (13, 14). Delayed diagnosis and therapy of acute staphylococcal osteomyelitis may be one factor responsible for progression to chronic osteomyelitis (1, 4). Failure to identify *S. aureus* as the cause of chronic osteomyelitis in patients with mixed cultures of sinus tract drainage or open wounds may result in inadequate therapy and treatment failure.

Serological tests measuring the antibody response to *S. aureus* could assist in identifying the etiological agent in osteomyelitis. In our patients with acute *S. aureus* osteomyelitis teichoic acid antibodies were elevated in 74%, whereas IgG antibodies to the ultrasonic extract were present in 52%. Nearly 80% of these patients were bacteremic. In chronic *S. aureus* osteomyelitis elevated titers of teichoic acid antibodies were present in 35% of our patients, whereas IgG antibodies were detected by radioimmunoassay in 47%.

False-positive results occurred only by radioimmunoassay. The radioimmunoassay is more sensitive than our immunodiffusion method, explaining its higher false-positivity rate. Infecting organisms in these patients included *P. aeruginosa* in four and *Enterococcus* sp., *Propionibacterium acnes*, *M. morgani*, and *Proteus mirabilis* in one patient each. Of the six controls with gram-negative osteomyelitis

and high levels of IgM or IgG antibodies to *S. aureus*, four were intravenous drug addicts and another was a diabetic with chronic foot infections, groups recognized to be at risk for staphylococcal skin colonization and infection (9, 10). Thus, positive results in these individuals were possibly caused by prior staphylococcal infection rather than by cross-reactivity due to shared antigens.

In conclusion, antibodies to a variety of staphylococcal antigens develop in 73% of patients with acute and 47% with chronic osteomyelitis caused by *S. aureus*. The serological response could be useful for identifying *S. aureus* as the etiological agent in acute osteomyelitis before blood culture results were known or in patients with negative cultures. Also, elevated staphylococcal antibodies in patients with *S. aureus* bacteremia would support a search for metastatic sites of infection in bone, since a 4- to 6-week course of parenteral antibiotic therapy would be necessary in these individuals (7, 15, 17). High levels of staphylococcal antibodies, particularly to teichoic acid, in patients with chronic osteomyelitis support active infection with *S. aureus*. This test may also be helpful in establishing an etiological diagnosis in patients with proven or suspected osteomyelitis with cultures of sinus tract drainage containing multiple organisms. Failure to isolate *S. aureus* from the sinus tract does not exclude it as the cause of chronic osteomyelitis, since sinus tract cultures may be falsely negative in proven staphylococcal osteomyelitis (5); the serological tests may be helpful in such individuals. Results of the current investigation justify prospective studies to test these hypotheses. However, teichoic acid antibodies may also result from chronic or extensive skin or soft-tissue infections (15). Thus, a positive result cannot be used to distinguish osteomyelitis from these other staphylococcal infections. Furthermore, negative results, seen in one-quarter of our patients with acute and two-thirds with chronic *S. aureus* osteomyelitis, do not exclude a staphylococcal etiology.

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