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Distinguishing Complicated from Uncomplicated Bacteremia Caused by *Staphylococcus aureus:* The Value of "New" and "Old" Serological Tests

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Antibody responses to staphylococcal α-toxin, cell wall teichoic acid, and cell wall peptidoglycan were measured in 259 serum samples from 74 consecutive patients with *Staphylococcus aureus* bacteremia. All patients with complicated bacteremia were seropositive in at least one of three tests, and 18 (72%) of 25 were positive in two or three assays; six (75%) of eight patients with endocarditis were positive for all three tests. In contrast, 15 (75%) of 20 patients with uncomplicated bacteremia were positive in only one or none of the tests. These differences in antibody response patterns were statistically significant ($\chi^2 = 18.33$, $P < .001$). Patients with complicated bacteremia had peak antibody titers that were significantly higher than those of patients with uncomplicated bacteremia. The assay for antibody to α-toxin was as sensitive as the assays for antibody to cell wall antigens but had less specificity for complicated bacteremia. The clinical severity of the bacteremia did not correlate with a complicated vs. uncomplicated nature of the infection but was predictive of early death due to staphylococcemia. The calculated predictive values suggest that the serology of *S. aureus* bacteremia may be clinically valuable when multiple tests are performed in paired serum samples.

Various serological tests have been proposed to help in the diagnosis and management of *Staphylococcus aureus* infections. Most of the earlier reports have focused on the use of the antitoxin assays, such as the tests for antibody to α-toxin and antibody to leucocidin, in the diagnosis of occult staphylococcal infections [1, 2]. Acute, deep infections, especially those affecting bone tissue, predictably yield increased levels of α-toxin– and leucocidin-neutralizing antibody in serum; chronic osteomyelitis and more superficially located infections, however, are less likely to do so [3–11]. Also, the need for serological tests to assist in the diagnosis of occult *S. aureus* infections has waned in favor of other techniques such as computer-assisted tomography, isotope scanning, and more invasive diagnostic procedures.

In recent years attention has been focused on the measurement of antibody to cell wall antigens in patients with proven *S. aureus* bacteremia. Teichoic acid and peptidoglycan are the major components of the staphylococcal cell wall, and they have been successfully tried as antigens in these newer assays [12, 13]. The assays have not been studied so much for their potential use as a diagnostic tool but for their possible use in the management of patients with proven *S. aureus* bacteremia. In this circumstance the serological assays are required to identify those patients who have a complicated bacteremic infection with deep foci, are at a high risk for metastatic spread, and therefore need higher doses of and prolonged treatment with antibiotics as well as a careful clinical follow-up [14–16]. However, the clinical value of the assay for antibody to teichoic acid has been seriously questioned by some authors [17–19], and even proponents of the assay caution against overemphasizing its value [15].

Furthermore, it is at present not clear whether the powers of the newer assays to distinguish patients with complicated bacteremia from those with uncomplicated bacteremia are significantly greater than those of the antitoxin assays used for so many years. Therefore, this study was designed to compare the antibody response to α-toxin, teichoic acid, and peptidoglycan in patients with complicated and uncomplicated infections. Over a 21-month period, all patients admitted to the Dijkzigt University Hospital with *S. aureus* bacteremia were entered into this prospective study.

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Patients and Methods

Patients. Seventy-seven consecutive patients with community-acquired ($n = 14$) or hospital-acquired ($n = 63$) *S. aureus* bacteremia admitted to the Dijkzigt University Hospital (Rotterdam, the Netherlands) were enrolled into the study. A total of 259 serum samples were collected from 74 patients. Twenty-one patients donated one serum sample only or were observed for less than one week or both. Fourteen of these patients died shortly after the onset of bacteremia; the remaining seven patients all had a clinically insignificant (see below) illness associated with a positive culture of blood and were discharged from the hospital within 10 days after this first positive culture. None of these patients had a complicated bacteremic infection. The other 53 patients were each followed up for at least one week (median, 33 days; range, 7–133 days). Each patient had at least two serum samples drawn (mean number of samples per patient, 4; range, 2–12); the average (± SD) rate of sampling was once every 9.6 ± 2.1 days.

In these 53 patients the median number of days between the probable onset of disease and the first positive culture of blood was 3 (range, 1–45 days); in 84% of the patients the sample giving the first positive culture was drawn within 10 days after the probable onset of disease. The median number of days between the first positive culture of blood and the first serum sampling was 4.5 days (range, −4–12 days); in three patients serum was stored from as early as four days before the first positive culture of blood. In 91% of the patients the first serum sample was collected within eight days after the first positive culture of blood. All cases of bacteremic infections were classified according to the criteria of Iannini and Crossley [20]: (*1*) uncomplicated bacteremia ($n = 33$), in which a removable focus of infection could be identified and was removed promptly; (*2*) complicated bacteremia ($n = 30$), in which a focus of infection was identified but could not be removed; and (*3*) bacteremia from an unknown source ($n = 14$), in which a focus of infection was not found. Among the 53 patients for whom there were multiple serum samples, complicated bacteremia was found in 25: eight with endocarditis, five with postoperative wound infection, five with lower-respiratory-tract infection, five with osteomyelitis or arthritis, and one each with an infected pancreatic abscess or an infected aortic balloon pump. Of the 20 patients with uncomplicated bacteremia, 18 had infected intravascular sites due to catheters, and two had furuncles.

Using a slight modification of the criteria described by Wilson et al. [21], we also classified all bacteremic episodes as clinically significant ($n = 36$) or not clinically significant ($n = 41$). Clinically significant cases of bacteremia consisted of features including two or more positive cultures of blood taken no more than four days apart, plus at least three of the following criteria: temperature ≥38.5 C, pulse rate ≥100/min, chills, leukocyte count $\geq 10 \times 10^9$/liter, hypotension (systolic blood pressure ≤90 mm Hg or a fall in pressure ≥30mm Hg), or oliguria (urine output ≤400 ml/24 hr without obstructive pathology). On the basis of these criteria, 29 (54.7%) of 53 patients that had multiple serum samples drawn experienced clinically significant bacteremia.

Paired serum samples, spaced eight to 10 days apart, were also obtained from 24 patients with bacteremia that was either complicated ($n = 9$), uncomplicated ($n = 11$), or of an unknown source ($n = 4$) and caused by gram-positive bacteria other than *S. aureus*. Cases of complicated bacteremia included patients with viridans streptococci causing subacute endocarditis ($n = 3$), enterococcus causing intraabdominal septicemia ($n = 2$), and *Proprionibacterium* causing an infected CSF shunt (1), *Streptococcus pneumoniae* causing infection from a CSF leakage (1), enterococcus causing sternal wound infection (1), and *Staphylococcus epidermidis* causing infection from a Hickman catheter left in situ for 10 days (1). Uncomplicated bacteremias, in all cases except one, were from infected iv lines that yielded *S. epidermidis* ($n = 7$), *Corynebacterium* spp. ($n = 1$), *S. epidermidis* plus diphtheroids ($n = 1$), or enterococcus ($n = 1$); the remaining patient in this group had group A hemolytic streptococcal bacteremia caused by an infected wound. Positive cultures of blood containing viridans streptococci ($n = 2$) and *S. epidermidis* plus diphtheroids ($n = 2$) were from patients with unknown foci of infection; multiple cultures of blood from 16 patients, including those with complicated bacteremia, yielded the same organism(s).

Antibody assays. The serum samples were assayed by ELISA for IgG antibody to teichoic acid and peptidoglycan of *S. aureus* cell wall, as previously described [13]; highly purified antigens were used in these assays (kindly donated by B. J. Wilkinson, University of Illinois, Normal, Ill). Neutralizing antibody to staphylococcal α-toxin was titrated by a standardized hemolytic assay (Behringwerke, Marburg, Federal Republic of Germany). Patients

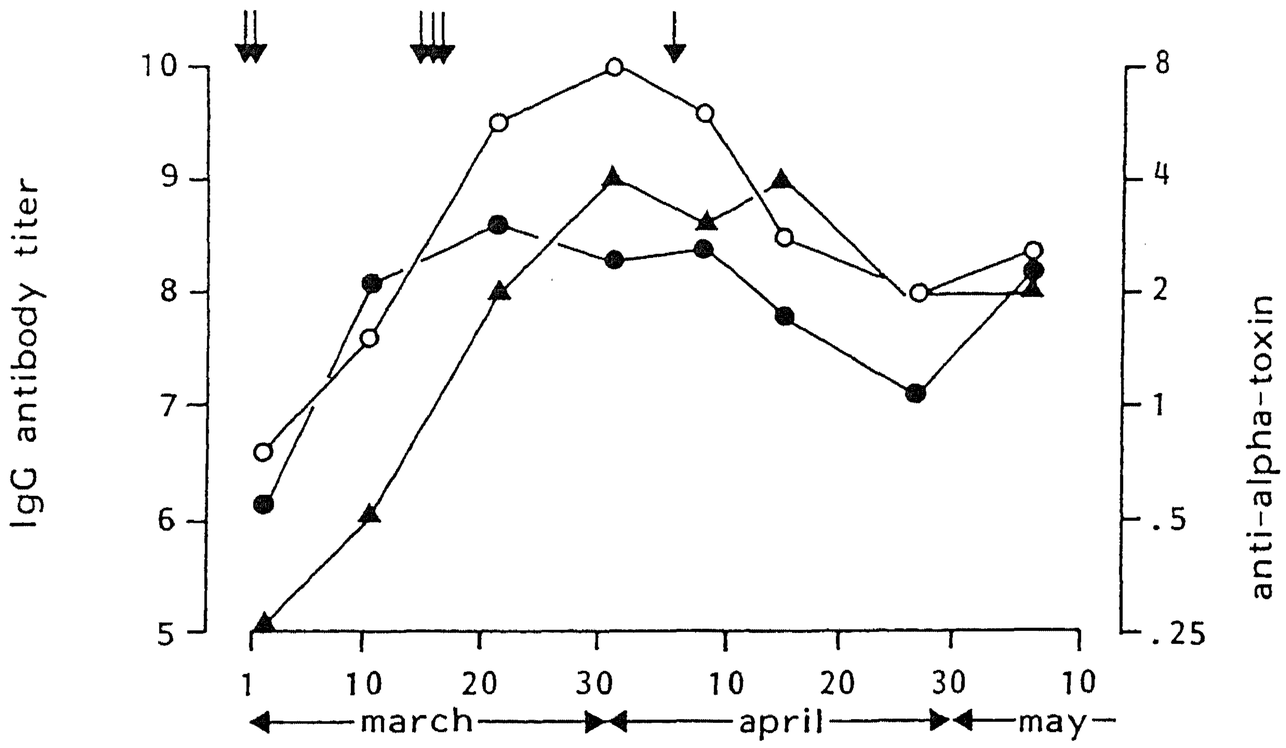

Figure 1. Kinetics of antibody response to α-toxin, teichoic acid, and peptidoglycan in a patient with *S. aureus* endocarditis. Arrows above figure indicate positive blood cultures. ○ = peptidoglycan; ● = teichoic acid; ▲ = α-toxin. IgG antibody titer is expressed as $\log_2$ serum dilution; antibody to α-toxin is expressed in IU/ml. Responses were determined from 1 March to 10 May 1982.

were considered to have a significant antibody response to these antigens (peptidoglycan and teichoic acid) when the titers of specific antibodies in their serum exceeded the average + 2 SD value of those of healthy controls or exceeded 2 IU of antibody to α-toxin. A $\geqslant$ 1.5 $\log_2$ unit increase in the level of antibodies to teichoic acid or peptidoglycan in serial serum samples was also considered a significant antibody response, as was a threefold change in the titer of antibody to α-toxin [22].

Statistics. Statistical analysis was performed by calculating significance levels of fourfold tables with continuity correction and by two-tailed tests of significance [23]. Means are expressed as the geometric mean.

Results

Of the 77 patients with *S. aureus* bacteremia, 14 (18%) died within seven days after the last positive culture of blood. Mortality was significantly correlated with the clinical severity of the bacteremic episode; 12 (33.3%) of 36 patients with clinically significant bacteremia died, but only 2 (4.9%) of 41 patients who had bacteremia that was not clinically significant died ($P < .005$). Mortality due to staphylococcemia, however, did not correlate with the nature of the underlying infection. Thus, patients with complicated and uncomplicated *S. aureus* bacteremia had similar mortalities (26.7 vs. 15.2%, respectively; $P > .1$).

Serological analysis was limited to those 53 patients who survived beyond the first postbacteremic week and who had at least two serum samples drawn seven days or more apart.

Serial serum sampling proved to be of paramount importance in detecting antibody responses in patients with *S. aureus* bacteremia. The first serum sample, taken within two weeks after the first positive culture of blood, yielded elevated titers of antibody to peptidoglycan, teichoic acid, and α-toxin in only 8 (15%), 5 (9%), and 9 (17%) of the 53 patients, respectively. Later serum samples demonstrated that 27 (51%), 23 (43%), and 30 (57%) of these patients had responded to the respective antigens. Thus, many patients significantly increased their specific antibody titers only after some time. The kinetics of the antibody responses in those patients with significant changes in titer are shown in figure 1 and table 1. Antibody titers initially doubled within one to two weeks (mean, six to seven days). Peak antibody titers were reached three to four weeks after the first positive culture of blood. The average total increase in titer was fourfold for antibody to teichoic acid, almost sixfold for antibody to peptidoglycan, and over ninefold for the neutralizing antibodies to α-toxin. However, the total increase varied considerably among these patients (table 1).

The results of the serological tests correlated well with the class of infection, i.e., complicated vs. uncomplicated *S. aureus* bacteremia (table 2). All patients with complicated bacteremia responded to at least one of the antigens used; 18 (72%) of these 25 patients were seropositive for at least two antigens, and 9 (36%) gave positive results in all three serological tests. Of eight cases of *S. aureus* endocarditis, 6 (75%) were among patients with positive serology for all three antigens. In contrast, 10 (50%) of the 20 patients with uncomplicated bacteremia responded to one antigen only, and 5 (25%) remained

Table 1. Kinetics of antibody response to peptidoglycan, teichoic acid, and α-toxin.

Antigen (no. of patients)	Initial rate of antibody response*	No. of days until peak antibody response†	Fold increase in antibody titer‡
Peptidoglycan (15)	6.9 (1.3–14)	34 (15–58)	5.9 (2.8–45.0)
Teichoic acid (13)	6.8 (3.2–11)	19 (11–29)	4.0 (3.0–7.0)
α-toxin (18)	6.0 (1.8–14)	25 (11–47)	9.2 (3.0–32.0)

* Doubling time in days (range).
† Measured from time of first positive culture of blood (range).
‡ Fold increase of peak titer from initial titer (range).

seronegative for all three antigens. The patterns of antibody responses of patients with complicated and uncomplicated *S. aureus* bacteremia were significantly different ($\chi^2 = 18.33$, 3 *df*, $P < .001$).

The peak titers of antibodies to peptidoglycan and teichoic acid were also significantly higher (more than twofold; $P < .05$) in patients with complicated bacteremia compared with those in patients with uncomplicated bacteremia (data not shown). Although the titers of antibody to α-toxin were on average almost twice as high in patients with complicated bacteremia, this difference did not reach statistical significance (data not shown). The highest titers of antibody against each of the three antigens were found in patients with *S. aureus* endocarditis.

The results of the serological tests did not correlate with the clinical severity of the bacteremia. Thus, patients who fulfilled the criteria for clinically significant bacteremia had antibody response patterns similar to those of patients with *S. aureus* bacteremia that was not clinically significant (table 3). Although seropositivity for all three antigens occurred more frequently in patients with clinically significant bacteremia than in patients with less severe bacteremic episodes (27.6% vs. 8.3%, respectively), this difference did not reach high levels of significance ($\chi^2 = 3.18$, $.05 < P < .1$). In addition, the peak antibody titer did not reach higher levels in patients with a clinically significant bacteremic episode (data not shown). Thus, the specific antibody responses of patients with *S. aureus* bacteremia are related to the nature of the underlying infection, i.e., complicated vs. uncomplicated diseases, but not to the clinical severity of the illness at the time of bacteremia.

The clinical usefulness of serological tests depends on their senisitivity and specificity, which at a known prevalence of the disease, can be translated into predictive values for a positive or negative test result. These values were calculated for the detection of complicated bacteremic infections among all patients with *S. aureus* bacteremia by using the three serological tests presented in this study (table 4). The values indicate that these tests may be helpful in identifying patients with a complicated bacteremic infection. Patients that remain seronegative for all three antigens can safely be considered free of complicated bacteremia, whereas those patients who are seropositive for all three antigens should be regarded as having a complicated infection with *S. aureus*. Many of the patients with *S. aureus* bacteremia will only be positive for one or two of the serological tests; in our study 37 (70%) of 53 patients fell into this category. When the cutoff is set at positivity for two or more tests, serology has positive and negative predictive values of 74% and 73%, respectively.

When each of the serological tests were separately

Table 2. Correlation of serological tests with class of *S. aureus* bacteremia.

Classification of bacteremia (*n*)	No. of patients seropositive for								
	None of the antigens (%)	One antigen				Two antigens				All three antigens (%)
		PG	TA	αT	%	PG + TA	PG + αT	TA + αT	%	
Uncomplicated (20)	5 (25)*	2	1	7	50	2	1	2	25	0
Complicated (25)	0	1	1	5	28	5	3	1	36	9 (36)†
Unknown (8)	1 (12.5)	2	2	1	62.5	1	0	0	12.5	1 (12.5)

NOTE. Antigens are abbreviated as follows: PG = peptidoglycan, TA = teichoic acid, and αT = α-toxin.
* Significantly more frequent than in complicated bacteremia ($\chi^2 = 7.03$, $P < .01$).
† Significantly more frequent than in uncomplicated bacteremia ($\chi^2 = 9.0$, $P < .01$).

Table 3. Failure of serological tests to correlate with clinical severity of *S. aureus* bacteremia.

Severity of bacteremia (*n*)	No. of patients seropositive for									
	None of the antigens (%)	One antigen				Two antigens				All three antigens (%)
		PG	TA	αT	%	PG + TA	PG + αT	TA + αT	%	
Not significant (24)	4 (16.7)	4	2	5	45.8	4	1	2	29.2	2 (8.3)
Significant (29)	4 (13.8)	2	0	7	31.0	4	3	1	27.6	8 (27.6)*

NOTE. Antigens are abbreviated as follows: PG = peptidoglycan, TA = teichoic acid, and αT = α-toxin.

* Compared with bacteremia that is not clinically significant, $\chi^2 = 3.18$; $0.05 < P < 0.1$.

analyzed for their ability to discriminate between complicated and uncomplicated bacteremia, the assay for antibody to peptidoglycan was the most sensitive (72%) and specific (73%) with positive and negative predictive values of 74% and 73%, respectively (at a prevalence of complicated bacteremia of 50%). The assay for antibody to teichoic acid was less sensitive (64%), and the assay for antibody to α-toxin had a much lower specifity (50%).

Antibodies cross-reacting with *S. aureus* peptidoglycan, teichoic acid, and α-toxin were found in 7 (29.2%), 2 (8.3%), and 1 (4.2%) of the control patients, respectively (table 5); they were present in five (55.6%) of nine patients with complicated bacteremia and in three (27.3%) of 11 patients with uncomplicated bacteremia due to gram-positive bacteria other than *S. aureus* ($P > .1$). Cross-reactions with peptidoglycan alone were seen in two patients with streptococcal endocarditis and in three patients with *S. epidermidis* septicemia due to infected iv lines; although these latter patients were classified as having uncomplicated bacteremia, removal of the infected lines was delayed seven or more days after the probable onset of disease. One patient with *S. epidermidis* bacteremia from an infected Hickman catheter showed increased titers of antibody cross-reacting with *S. aureus* teichoic acid only. Two patients showed cross-reactive antibody to two *S. aureus* antigens: one patient with streptococcal endocarditis had high titers of antibody to peptidoglycan and antibody to teichoic acid, and one patient with abdominal enterococcus septicemia showed cross-reactions with *S. aureus* peptidoglycan and α-toxin. None of the patients' sera cross-reacted with all three *S. aureus* antigens (table 5).

Discussion

The primary goal of this study was to investigate the ability of old and new serological tests to discriminate between complicated and uncomplicated infection in patients with *S. aureus* bacteremia. No single test proved able to detect complicated bacteremia in all patients, nor was there a test that was totally specific in this respect. Sensitivities ranged from 64% for assays of antibody to teichoic acid to 72% for assays of antibody to peptidoglycan and α-toxin. The specificity of assays for antibody to cell wall antigens (peptidoglycan and teichoic acid) was 75%, whereas this specificity was 50% for α-toxin. Thus, the oldest test in staphylococcal serology, the assay for hemolytic antibody to α-toxin, proved to be as sensitive as the newer assays for antibodies to cell wall antigens, but was less specific for complicated *S. aureus* bacteremia.

The lack of high sensitivity and specificity has been noted repeatedly in the earlier studies of antibody to α-toxin in occult *S. aureus* infections [1, 3, 4, 6, 8, 9]. However, this problem has also been a disappointing reality of the newer assays of antibody

Table 4. Sensitivity, specificity, and predictive values of testing for antibodies to peptidoglycan, teichoic acid, and α-toxin.

Patients with complicated bacteremia seropositive for	Test sensitivity (%)	Test specificity (%)	Predictive value (%) of*	
			Positive result	Negative result
Three antigens	36	100	100	61
At least two antigens	72	75	74	73
At least one antigen	100	25	57	100

* Predictive values were calculated at a prevalence = 50%.

Table 5. Cross-reactive antibodies to *S. aureus* in control patients with gram-positive bacteremia.

Classification of bacteremia (*n*)	No. of patients seropositive for								
	None of the antigens (%)	One antigen				Two antigens			
		PG	TA	αT	%	PG + TA	PG + αT	TA + αT	%
Uncomplicated (11)	8 (72.7)	3	0	0	27.3	0	0	0	-
Complicated (9)	4 (44.4)	2	1	0	33.3	1	1	0	22.2

NOTE. None of the patients were seropositive for all three antigens. The four patients with bacteremia from an unknown source were not seropositive for any of the antigens. The antigens are abbreviated as follows: PG = peptidoglycan, TA = teichoic acid, and αT = α-toxin.

to cell wall in patients with bacteremic infections due to this organism [13–15, 24, 25]. Patients with complicated bacteremia, even those that undergo the intense immunogenic stimulus of *S. aureus* endocarditis, may remain negative in any one test. This is certainly not due to an inability of the recently developed ELISA or RIA to detect low levels of specific antibody; in contrast, these newer techniques will detect specific antibody in virtually all sera from healthy donors as well. When serial serum samples are tested, as was done in the present study, even small changes in specific titers of antibody can be measured [22, 26]. Still, patients can have complicated bacteremia but not react in a given assay. However, when more than a single serological test is performed, more patients show a significant antibody response to at least one of the antigens used.

In this study, all patients with a complicated bacteremic infection were seropositive in at least one of the three serological assays. In this group of patients, those with *S. aureus* endocarditis usually were positive for all three tests and had higher titers of antibody, whereas patients with other forms of complicated bacteremia were positive in only one or two of the tests. Such an increase in the number of seropositive patients has also been noted in previous studies of antitoxin and has led some authors to recommend performing at least two serological tests for patients with diseases caused by *S. aureus* [1, 6, 9]. Results of this study clearly support the need for two or three different serological assays in *S. aureus* bacteremia. Our findings also stress the need for testing paired serum samples in a patient with this bacteremia; one serum should be taken as soon as staphylococcemia is detected, and one two weeks later. If only a single serum sample is taken at two weeks, a sizable proportion of seropositive patients may be missed; 12%–21% of the patients in this study had a significant rise in levels of specific antibody that did not boost the final peak titer into an elevated range compared with results from healthy controls. Only few studies have serially measured levels of antibody to α-toxin in patients with *S. aureus* diseases. Simon [8] detected 90% of deep-seated infections only when serial determinations of the levels of antibody to α-toxin were done. More limited studies by Christensson et al. [26], Julander et al. [27], and Verbrugh et al. [22] have provided evidence that the sensitivity of serological studies of various *S. aureus* infections with the newer assays for antibody to cell wall antigen is much improved if adequately spaced serum samples are tested.

Interestingly, the clinical impression of the bacteremic episode did not correlate with the complicated or uncomplicated nature of the infection. Thus, patients with a clinically severe bacteremic episode could very well have an uncomplicated bacteremic infection, e.g., one induced by a readily removable intravascular device. Eleven (37.9%) of 29 patients with clinically significant bacteremia in this study had an uncomplicated infection. The clinical severity score was, however, predictive of early death associated with the staphylococcemia. The cross-reactions that were found in this study among control patients with gram-positive bacteremia other than *S. aureus* would argue against using these assays for the diagnosis of culture-negative *S. aureus* infections. Such cross-reactions or false-positive results, especially in assays for antibody to cell wall antigen, have been noted before and are best explained by the ubiquity of peptidoglycans and teichoic acids in the cell walls of many species of gram-positive bacteria [12, 25]. The assay for antibody to α-toxin is much more specific for *S. aureus*

diseases and may, therefore, have a greater potential as part of a diagnostic protocol of hidden *S. aureus* infection.

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