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Δ APACHE II for Predicting Course and Outcome of Nosocomial *Staphylococcus aureus* Bacteremia and Its Relation to Host Defense

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To predict at an early phase the clinical course and outcome of nosocomial *Staphylococcus aureus* bacteremia, the APACHE II score at onset of bacteremia was calculated in 99 patients. A Δ APACHE II score (i.e., the difference between this score and one calculated for the day before the bacteremia) was also determined. This Δ APACHE II score was highly significantly correlated with clinical course ($P < .001$) and outcome ($P < .001$). The risk of a complicated clinical course and of dying from *S. aureus* bacteremia is determined at the very onset of bacteremia, and this risk can best be assessed by calculating the Δ APACHE II score. Furthermore, a positive correlation was found between Δ APACHE II scores and the level of serum opsonic activity ($P = .003$) toward *S. aureus*. Therefore, the complicated clinical course of *S. aureus* bacteremia is not due to a relative lack of specific opsonins.

In contrast to gram-negative septicemia, bacteremic infection with *Staphylococcus aureus* infrequently leads to acute life-threatening situations such as shock or multiple organ failure [1]. The severity of *S. aureus* bacteremia is determined much more by complications resulting from the development of metastatic foci of infection [2]. In uncomplicated cases of bacteremia, a short (2-week) course of antistaphylococcal therapy after removal of an identified primary focus of infection will usually be sufficient [2]. However, in cases with metastatic foci, 4–6 weeks of intravenous therapy with high doses of antistaphylococcal antibiotics is recommended to overcome the infection [2].

At present, objective parameters to predict, in an early phase, the likelihood of development of metastatic foci or the risk of death due to the infection have not been described. Serology has proven to be of predictive value but only after progression of the infection has allowed an antibody response [3]. An accurate estimate of the chance of progression to a complicated or life-threatening condition at the time of *S. aureus* bacteremia may well be of clinical value. Therefore, in patients who developed *S. aureus* bacteremia, we compared APACHE II scores [4] determined the day before bacteremia and on the day of onset to evaluate the scores as predictors of clinical course and

outcome. Host defense was investigated to determine whether, at the time of presentation, the severity of *S. aureus* bacteremia depended on the quality of the opsonic system. Since phagocytic cell functions are rarely impaired [5], attention was focused primarily on humoral serum factors.

Patients and Methods

Patients. During a 3-year period, all patients in University Hospital Rotterdam with blood cultures yielding *S. aureus* were followed. A distinction was made between patients with community-acquired and those with hospital-acquired bacteremia according to CDC criteria [6]. Only patients with hospital-acquired bacteremia ($n = 104$) were included in this study. For these patients, the presence of underlying diseases and the reason for admission were noted. If surgery was done before the bacteremia, this was also noted, as was stay in an intensive care unit. Antibiotic treatment was recorded and scored as adequate, adequate only after modification, inadequate, or no therapy. In each case, an effort was made to establish a primary focus of infection. A focus was recorded as proven by culture or, in the absence of a culture, as probable. Cases in which no focus could be recognized were scored as focus unknown. After discharge, patients were followed for 1 year in which possible late-onset complications of bacteremia were registered.

Course and outcome of the bacteremia. The course of each episode of bacteremia was scored as either complicated or uncomplicated. Bacteremia was considered uncomplicated if there were no signs of septic shock and if, besides the primary focus of infection, no evidence of metastasis to distant foci was present. In a case with a removed intravascular device, the presence of a local abscess or infiltration restricted to the insertion site was also considered an uncomplicated infection. The course of the bacteremia was considered complicated in cases with septic shock at onset of bacteremia and in cases of suppurative metastasis later in the disease course. Septic shock was defined as hypotension that needed treatment with inotropic agents.

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Informed consent was obtained from all patients or their guardians for blood sampling in addition to diagnostic blood sampling. Human experimentation guidelines of University Hospital Rotterdam were followed.

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For evaluation of outcome, all episodes of bacteremia were also classified according to the following categories: Cure was survival with disappearance of all clinical signs of the bacteremia. Improvement was survival with a significant clinical improvement but without complete disappearance of symptoms that could be attributed to the infection. Failure was death due to the infection. If a patient died within 48 h after onset of the bacteremia, it was classified as an early death. All other deaths were considered late.

Patients were scored according to the APACHE II severity of disease classification system, which is a well-known scoring system based on chronic health points (age and underlying diseases) and on an acute physiology score [4]. The physiologic parameters were scored twice, once on the day the first blood culture positive for *S. aureus* was taken and once from data obtained the day before. Of 59 patients who were not in an intensive care unit at the onset of bacteremia, missing values of oxygenation and of the Glasgow Coma Score for 41 were assumed to be normal on the basis of clinical evidence. Of the 104 patients, 5 were considered nonevaluable because of lack of information about therapy, course, and outcome or because of missing values needed to calculate an APACHE II score.

Δ APACHE II score. Δ APACHE II score was defined as the APACHE II score on the day the first positive blood culture was taken minus the score from the day before. The difference between these two scores was regarded to be the result of the intervening bacteremia and corrects for the variability in the prebacteremic condition of the patients.

Blood samples. From 28 patients, blood samples were obtained as soon as possible after their first positive blood culture for *S. aureus* and, if possible, 1 week later. Sera were stored within 30 min after collection at -70°C . The sera were used in the phagocytosis assay, with pooled human serum (PHS) from 8 healthy volunteers as controls.

***Staphylococcus aureus* strains.** After primary isolation from blood, the *S. aureus* strains were lyophilized to ensure conservation of the wild type cell wall structures. As the control strain for phagocytosis, we used a clinical *S. aureus* isolate with an intermediate uptake after opsonization in PHS.

Phagocytosis assay. Phagocytosis was determined by a modification of the method described by Verbrugh et al [7]. Human polymorphonuclear leukocytes (PMNL) were isolated from fresh citrate anti-coagulated donor blood, adjusted in Hanks' balanced salt solution (HBSS) with 1% gelatin and 5% fetal calf serum to a concentration of 1.10^7 PMNL/mL, and kept on ice until phagocytosis was started. The [^3H]thymidine-labeled (Amersham International, Amersham, UK) *S. aureus* strains were preopsonized with heated (30 min, 56°C) and freshly thawed sera, diluted 1/20 in HBSS, by incubation at 37°C for 15 min. After centrifugation, the opsonized bacteria were resuspended in HBSS (5.10^8 cfu/mL) and kept on ice. Phagocytosis was started by constituting mixtures of 0.5 mL of PMNL, 0.1 mL of opsonized bacteria, and 0.4 mL of HBSS. After 10 min, phagocytosis was stopped with ice-cold PBS. Extracellular bacteria were removed by centrifugation, and leukocyte-adherent bacteria were lysed with lysostaphin (2 mg/L, Sigma, St. Louis) for 10 min at 4°C . Phagocytosis was expressed as the percentage of radioactivity remaining with the leukocyte fraction, determined by liquid scintillation counting.

For the following opsonic mixtures, uptake was measured: control strain opsonized in PHS, control strain opsonized in patient serum, patient strain opsonized in PHS, and patient strain opsonized in patient serum. To allow more meaningful comparison of the serum opsonic activity of individual patients, differences due to variability in opsonic requirements of different patients' strains were controlled by subtracting the uptake of a patient's strain with PHS from the uptake with the matched patient's serum.

Intracellular killing. All *S. aureus* strains isolated were tested for their capacity to resist intracellular killing by PMNL. The bacteria were opsonized in PHS. After 3 min of phagocytosis (see above), all extracellular bacteria were removed by centrifugation and lysostaphin treatment. The leukocytes were resuspended in HBSS with 5% PHS and incubated at 37°C . Intracellular killing was measured by comparing the amount of viable intracellular bacteria at 0, 15, 30, 45, and 60 min.

Statistical evaluation. The results were analyzed using the SAS statistical package [8]. Multivariate logistic regression analysis was applied to determine the contribution of individual variables on course (uncomplicated vs. complicated) and outcome (survived vs. died), respectively. Backward elimination was used to determine the significance of these variables. With the same procedure, the contribution of the individual variables of Δ APACHE II were determined. Differences between groups were determined by the *t* test, the Mann-Whitney *U* test, or Fisher's exact test, as appropriate. Positive and negative predictive values as well as relative risks were calculated for Δ APACHE II scores of ≥ 7 points or < 7 points. Correlations between Δ APACHE II score and phagocytosis were determined with Spearman's rank test. This test was also used for comparing serially collected sera. The Wilcoxon matched-pairs test was used to compare the opsonic response against the control strain to the response against patient isolates. Significance was $P = .05$ (two-tailed test).

Results

The mean age of the 99 evaluable patients with hospital-acquired bacteremia was 54 years. Fifty patients (51%) had a history of cardiovascular disease; diabetes mellitus was found in 14 cases (14%) and renal disorders in 12 (12%). Other underlying diseases were of minor relevance. Of the 99 patients, 35 (35%) underwent surgery prior to the infection; 40 patients (40%) were in an intensive care unit at the onset of bacteremia.

Eleven (11%) of the 99 patients presented with shock. Six of them died early, in the acute phase of the septic shock episode; 4 patients died later in the disease course, 2 of them after developing endocarditis; 1 patient survived. Seven other patients presented with a decrease in blood pressure that was correctable with fluids only; 1 patient died. Of the 99 patients, 17 (17%) developed metastatic foci: 6 developed endocarditis, according to Von Reyn criteria [9], and 11 acquired foci at different sites, with suspicion of endocarditis in 3 cases. Eleven of these 17 patients died, 3 after surviving an initial period of shock. Of the 99 patients, 74 (75%) patients had an uncompli-

Table 1. Distribution of APACHE II scores before and at onset of nosocomial *S. aureus* bacteremia and their relation to complicated course or fatal outcome.

APACHE II score	Day before onset			Day of onset		
	Patients	Complicated only	Complicated and fatal	Patients	Complicated only	Complicated and fatal
0-3	14	1 (7)	1 (7)	2	0	0
4-6	29	4 (14)	3 (10)	8	0	0
7-9	22	1 (5)	6 (27)	22	1 (5)	0
10-12	19	0	5 (26)	19	1 (5)	1 (5)
13-15	10	0	2 (20)	21	2 (10)	5 (24)
16-18	3	1 (33)	1 (33)	14	1 (7)	7 (50)
19-21	2	0	0	9	1 (11)	3 (33)
≥22	0	0	0	4	1 (25)	2 (50)

NOTE. Data are no. (%) of patients.

cated clinical course and survived. The overall mortality rate was 18% (18/99). Of the 81 surviving patients, 69 (85%) were cured and 12 (15%) only improved after their bacteremic episode. No patient was readmitted for recurrence of *S. aureus* infection.

Antibiotic therapy. Of the 99 patients, 89 received adequate therapy shortly after onset of the bacteremic episode. Of the 18 patients who died, 15 had been given proper antimicrobial therapy.

Primary foci of infection and clinical course. If known, the primary foci of infection were documented. The most important foci were intravascular devices (59/99, 60%) and surgical wounds (22/99, 22%). Other bacteremias were of miscellaneous origin (4/99, 4%) or had no identifiable focus (14/99, 14%). There was no correlation found between the focus of infection and course or outcome of bacteremia, although 8 (36%) of 22 patients with a wound as the primary focus had a complicated disease course.

Relationships between calculated APACHE II scores and course. The relationships between the calculated APACHE II scores and the development of metastatic foci and outcome of infection are shown in table 1. In table 2, these relationships are shown for the Δ APACHE II score. There was no statisti-

cally significant relationship between the APACHE II score from the day before bacteremia onset and a complicated course or fatal outcome. In sharp contrast, the APACHE II score calculated on the day of bacteremia onset correlated well with clinical course ($P = .001$) and with outcome of infection ($P = .001$). Clearly, a large shift toward higher scores is observed in comparing APACHE II scores from the day before onset of bacteremia with those from the day of onset (table 1).

The calculated Δ APACHE II scores proved to correlate even better with both the rate of complicated clinical course ($P < .001$) and the mortality rate ($P < .001$; table 2). From this analysis, it is evident that patients who show an increase in the APACHE II score of ≥ 7 points early in an episode of *S. aureus* bacteremia have a much higher risk of having a complicated course of infection and are much more likely to die as a consequence.

Predictive values and relative risks. To substantiate the above-mentioned risk, the positive and negative predictive values for fatal outcome and complicated course were calculated for increases in Δ APACHE II scores of ≥ 7 points versus < 7 points. Using the observed prevalences in our study, the positive predictive value of a Δ APACHE II ≥ 7 was 58% (14/24) for a fatal outcome and 83% (20/24) for a complicated clinical course; the negative predictive values for scores < 7 were as high as 95% (71/75) for survival and 93% (70/75) for an uncomplicated course. Expressed in terms of relative risk (RR), a patient with a Δ APACHE II ≥ 7 had an RR for a complicated course of 12.5 (95% confidence interval [CI], 5.3%–29.7%) and an RR for a fatal outcome of 10.9 (95% CI, 4.0%–30.1%) compared with a patient with a score of < 7 points.

Multivariate analysis. To further investigate the relative merits of Δ APACHE II, a multivariate logistic regression analysis was carried out for the different APACHE II scores, age, sex, underlying diseases, focus of infection, and therapy. As a predictive parameter for a complicated course and fatal outcome, the Δ APACHE II score was the single most important

Table 2. Relationship between Δ APACHE II and clinical course and outcome in patients with nosocomial *S. aureus* bacteremia.

Δ APACHE II score*	Patients	Complicated only	Complicated and fatal
-2 to 3	40	0	2 (5)
4-6	35	1 (3)	2 (6)
7-9	16	5 (31)	7 (44)
≥10	8	1 (13)	7 (88)

NOTE. Data are no. (%) of patients.

* APACHE II score on day of onset minus APACHE II score 1 day earlier.

factor. None of the other parameters had additional value. A multivariate logistic analysis was also done to determine the significance of each of the physiologic variables included in the APACHE II system. For predicting mortality and a complicated course, mean arterial pressure proved to be the most significant variable. Changes in serum electrolytes (sodium, potassium) also contributed importantly to the predictive power of Δ -APACHE II.

Phagocytosis and intracellular killing. Phagocytosis of the individual *S. aureus* strains of 28 patients was tested after opsonization by PHS. No relation was found between Δ -APACHE II scores and uptake percentages after opsonization by PHS ($r = .08, P > .05$). Thus, strains that caused a more serious disease were not more resistant to phagocytosis after opsonization in PHS than were other strains. Moreover, all 28 strains were killed by PMNL (>90% within 60 min).

The Δ APACHE II scores of patients correlated positively with a higher level of opsonic activity in their sera, so the sickest patients had the highest *S. aureus* opsonic activity. This correlation was valid for *S. aureus* opsonic activity as tested with the patient's own strain ($r = .57, P = .003$; figure 1) and with the control strain ($r = .54, P = .005$, data not shown). Furthermore, a comparison between the patient strain and the control strain showed that opsonic activities were comparable

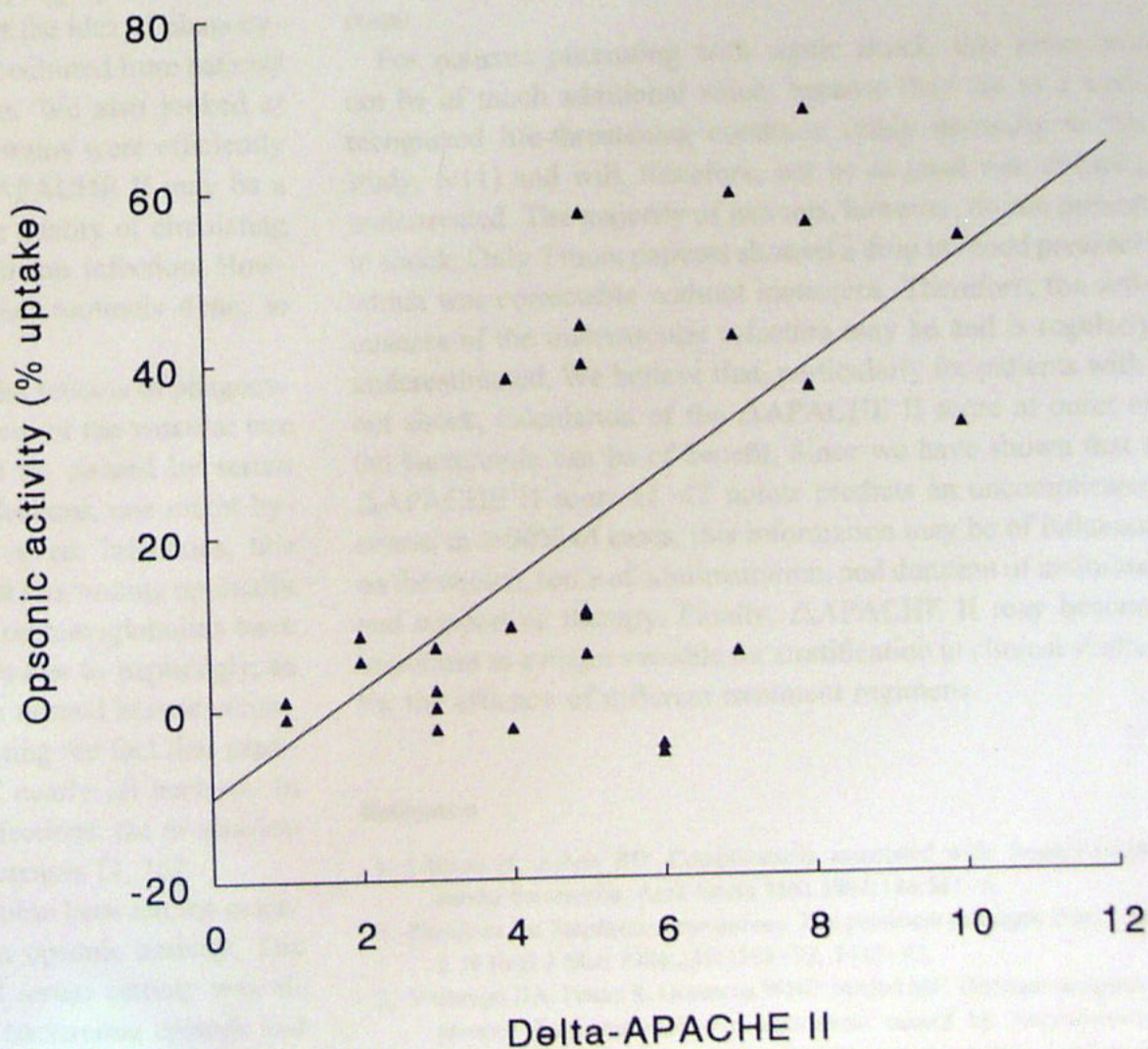
($r = .56, P = .004$, data not shown). After sera were heated, these correlations remained intact. There was good correlation between opsonic activities in the first and second sera ($r = .82, P < .002$). The level of the *S. aureus* opsonic activity in second sera was highly similar to that in first sera.

Discussion

The APACHE II classification system of severity of disease was predictive for both clinical course and final outcome in patients with *S. aureus* bacteremia. The predictive values were greatest if changes in the APACHE II score could be calculated by comparing the score from immediately before the onset of bacteremia with that after *S. aureus* invasion into the bloodstream. This Δ APACHE II score had predictive power for a complicated versus uncomplicated course of infection and for whether the patient survived the bacteremic episode.

The APACHE II score calculated for the day before onset of infection can be considered as an assessment of a patient's prebacteremic condition. From the literature [10], one would expect that a higher prebacteremic APACHE II score would be associated with a greater risk of dying as a consequence of the superimposed *S. aureus* bloodstream infection. However, this hypothesis was not confirmed in our study. In contrast, the

Figure 1. Correlation between Δ -APACHE II scores and opsonic activity of patient sera; patient's own strains were used. % uptake of *S. aureus* by PMNL is net difference: uptake of patients' serum-opsonized bacteria minus pooled human serum-opsonized bacteria. Spearman's correlation coefficient, $r = .57, P = .003$.



very good correlation between the increase in the APACHE II score from before onset to onset of *S. aureus* bacteremia and clinical course and outcome of the infection leads to the conclusion that the risk of developing metastatic foci and dying is largely determined at the very moment of bloodstream invasion and that this risk can be estimated from the seriousness of the presenting signs.

Since there seems to be no clear correlation between the prebacteremic health status of the patient and the symptoms of bacteremia, one might speculate that these symptoms of bacteremia most likely are the result of differences in the properties of the infecting strains [11]. In cases with shock (11% of our cases), this may well be the *S. aureus* property to be able to produce multiple extracellular toxins. Evidence for a role of specific differences in virulence of *S. aureus* strains in determining clinical course, however, remains poor. Capsulated bacteria are known to resist phagocytosis, and it might be expected that the more serious infections were caused by bacteria resisting recognition, ingestion, or killing by phagocytes. Capsular polysaccharides, protein A, and peptidoglycan of *S. aureus* have all been shown to inhibit phagocytosis in vitro [12]. However, our results do not support the idea of phagocytosis-resistant strains being preferentially cultured from patients who have a serious *S. aureus* infection. We also looked at intracellular killing by PMNL, but all strains were efficiently killed within 1 h. Alternatively, the Δ APACHE II may be a reflection of the quantity rather than the quality of circulating bacteria at the beginning of the bloodstream infection. However, quantitative blood cultures were not routinely done, so this statement remains speculative.

Host defense was also investigated. The process of phagocytosis is the most important defense system of the vascular tree against circulating bacteria that cannot be cleared by serum killing [13]. Therefore, in *S. aureus* infections, one might hypothesize that, in patients with more severe infections, this phagocytosis clearance mechanism is not functioning optimally [14]. Components of complement and immunoglobulins have opsonic capacity. For *S. aureus*, antibodies to peptidoglycan seem of primary importance [3, 15]. In normal human serum, these antibodies can be detected, reflecting the fact that peptidoglycan is a cell wall component of nearly all bacteria. In patients with complicated *S. aureus* infections, the production of antibodies against peptidoglycan increases [3, 16].

Our results showed a positive correlation between the calculated Δ APACHE II scores and serum opsonic activity. The results also showed that this increased serum activity was already present in an early phase of the bacteremic episode and remained detectable after sera were heated, suggesting that the activity is indeed (partially) antibody-related. The high level in serum opsonic activity might be due to a rapid booster response. It is important to notice that, despite the increased serum opsonic activity in the patients with a higher Δ APACHE II score, the risk of a complicated clinical course in these

patients was clearly greater than in patients with lower Δ -APACHE II scores. Obviously, the presence of a high level of serum opsonic activity for *S. aureus* is not protective against the development of more serious sequelae. Rather, the opsonic response in seriously ill patients seems to be a marker of the severity of disease. It also would be fascinating to know more about the patients' own PMNL. Therefore, for 7 of our patients who recovered from bacteremia with Δ APACHE II scores ranging from 3 to 8, routine leukocyte function (chemotaxis, phagocytosis, and intracellular killing) analysis was done 1 year later. None of these functions were impaired (data not presented).

The primary intention of our study, however, was not to evaluate the importance of possible risk factors or to elucidate the role of host defense factors but to present to the clinician a predictive tool to judge objectively the seriousness of a bacteremic episode with *S. aureus*. On the basis of our evaluations, we conclude that determination of the Δ APACHE II score for patients with positive blood cultures for *S. aureus* can be of great clinical value, as it provides an early assessment of the risk of a complicated clinical course and an unfavorable outcome.

For patients presenting with septic shock, this score will not be of much additional value, because they are in a well-recognized life-threatening condition (early mortality in this study, 6/11) and will, therefore, not be at great risk of being undertreated. The majority of patients, however, do not present in shock. Only 7 more patients showed a drop in blood pressure, which was correctable without inotropics. Therefore, the seriousness of the intravascular infection may be and is regularly underestimated. We believe that, particularly for patients without shock, calculation of the Δ APACHE II score at onset of the bacteremia can be of benefit. Since we have shown that a Δ APACHE II score of <7 points predicts an uncomplicated course in $>90\%$ of cases, this information may be of influence on the choice, route of administration, and duration of antibiotic and supportive therapy. Finally, Δ APACHE II may become important as a major variable for stratification in clinical studies for the efficacy of different treatment regimens.

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