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Increased Mortality with Accessory Gene Regulator (*agr*) Dysfunction in *Staphylococcus aureus* among Bacteremic Patients▽†

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Accessory gene regulator (*agr*) dysfunction in *Staphylococcus aureus* has been associated with a longer duration of bacteremia. We aimed to assess the independent association between *agr* dysfunction in *S. aureus* bacteremia and 30-day in-hospital mortality. This retrospective cohort study included all adult inpatients with *S. aureus* bacteremia admitted between 1 January 2003 and 30 June 2007. Severity of illness prior to culture collection was measured using the modified acute physiology score (APS). *agr* dysfunction in *S. aureus* was identified semiquantitatively by using a δ-hemolysin production assay. Cox proportional hazard models were used to measure the association between *agr* dysfunction and 30-day in-hospital mortality, statistically adjusting for patient and pathogen characteristics. Among 814 patient admissions complicated by *S. aureus* bacteremia, 181 (22%) patients were infected with *S. aureus* isolates with *agr* dysfunction. Overall, 18% of patients with *agr* dysfunction in *S. aureus* died, compared to 12% of those with functional *agr* in *S. aureus* ($P = 0.03$). There was a trend toward higher mortality among patients with *S. aureus* with *agr* dysfunction (adjusted hazard ratio [HR], 1.34; 95% confidence interval [CI], 0.87 to 2.06). Among patients with the highest APS (scores of >28), *agr* dysfunction in *S. aureus* was significantly associated with mortality (adjusted HR, 1.82; 95% CI, 1.03 to 3.21). This is the first study to demonstrate an independent association between *agr* dysfunction and mortality among severely ill patients. The δ-hemolysin assay examining *agr* function may be a simple and inexpensive approach to predicting patient outcomes and potentially optimizing antibiotic therapy.

Staphylococcus aureus is the second most common pathogen in health care-associated infections, responsible for 15% of all health care-associated infections reported to the National Healthcare Safety Network from 2006 to 2007 (13). *S. aureus* bacteremia is associated with high patient morbidity and mortality and confers a considerable financial burden to an already overwhelmed health care system (4, 5). Currently, many clinical laboratories devote significant resources to the rapid detection of the *S. aureus mecA* gene, which confers resistance to beta-lactam antibiotics, in an attempt to improve patient outcomes. However, other genetic elements of *S. aureus* may have similar clinical and epidemiologic importance.

The accessory gene regulator (*agr*) locus of *S. aureus* is a quorum-sensing, global regulon that coordinates the expression of dozens of housekeeping genes as well as the expression of secreted and cell-associated virulence factors (7, 37). Mutations causing *agr* dysfunction in the *agr* locus confer changes in the expression of autolysins and hemolysins and have global effects on bacterial phenotypes, including pathogenicity (8, 26, 34, 38).

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S. aureus agr dysfunction has been associated with a longer duration of bacteremia and with attenuated vancomycin bactericidal activity (7, 25, 27). The development of the vancomycin-intermediate *S. aureus* (VISA) phenotype has been accompanied by a marked reduction or complete loss of *agr* function, both through loss-of-function mutations in *agr* and through upstream suppression of *agr* expression (23, 25, 27, 34). *agr* knockout mutants have also demonstrated an increased proclivity to develop the VISA phenotype (7, 27, 37). *In vitro* studies have demonstrated that inactivation of *agr* attenuates the bactericidal activity of vancomycin, and certain clones of *S. aureus* with dysfunctional *agr* may have a survival advantage under vancomycin selective pressure, possibly related to decreased autolysis (26, 27). Studies of nosocomial bacteremia have shown that infection with *S. aureus* without *agr* function was more common among patients with persistent or prolonged *S. aureus* bacteremia than among patients with resolving *S. aureus* bacteremia (7, 21).

To our knowledge, no study has measured the independent association between *agr* dysfunction and mortality among *S. aureus*-infected patients. The purpose of this study was to assess the association between *agr* dysfunction and mortality in a large cohort of patients with *S. aureus* bacteremia while statistically adjusting for important patient characteristics, such as comorbid conditions and severity of illness, and pathogen phenotypes and genotypes.

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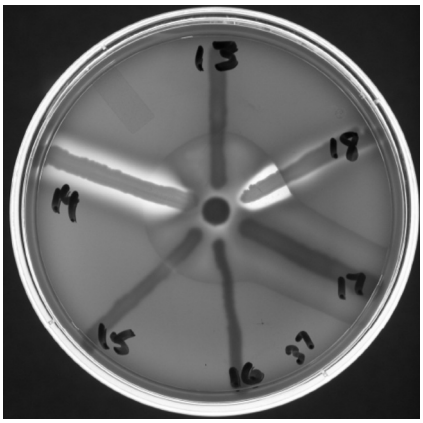

FIG. 1. Measurement of *agr* dysfunction by δ-hemolysin assay. Isolates were streaked near a β-hemolysin disk in order to test for δ-hemolysin production.

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MATERIALS AND METHODS

Study design and patient population. This retrospective cohort study included all adult admissions to the University of Maryland Medical Center between 1 January 2003 and 30 June 2007 with a positive blood culture for *S. aureus*. Each admission was treated as an independent event, and therefore patients may have been included in the study more than once. The first positive *S. aureus* blood culture from each admission was saved by the clinical microbiology laboratory for research purposes and used for this study. Eligible patients were identified using a relational database containing medical, pharmaceutical, and microbiologic data. This database has been validated in previous studies and has positive and negative predictive values in excess of 99% compared to paper medical records (9, 10, 18, 19, 22). The relational database was compared to paper medical records for 236 (29%) patients in our study cohort and was found to have positive predictive values of at least 97% and negative predictive values of at least 98%.

Additional variables that were not available in the relational database (e.g., components of the modified acute physiology score [APS]) were collected by a research nurse via chart review. This study was approved by the institutional review board of the University of Maryland, Baltimore, MD. A waiver of consent was granted given the retrospective nature of the project.

Laboratory methods. (i) Clinical strains. All *S. aureus*-positive blood cultures during the study period were initially identified by the clinical microbiology laboratory and were stored at −80°C. Identification of *S. aureus* was determined by Gram stain, colony morphology, and catalase- and coagulase-positive reactions.

(ii) *agr* phenotype and genotype studies. We operationalized *agr* dysfunction by using the level of δ-hemolysin production. This is the standard approach for the measurement of *agr* function because RNAIII, the effector of *agr* regulation, encodes δ-hemolysin (34). δ-Hemolysin production was measured by streaking the *S. aureus* isolate adjacent to a β-hemolysin disk (Remel, Lenexa, KS) on a tryptic soy agar plate with 5% sheep blood, incubating it at 37°C overnight, and evaluating it for synergistic hemolysis within the β-hemolysin zone produced by the disk containing bacterial growth. The presence of synergistic hemolysis within the β-hemolysin zone indicates the production of δ-hemolysin by the test organism and, therefore, a functional *agr* locus (27). *agr* dysfunction was defined as the complete absence of δ-hemolysin within the β-hemolysin zone, as evidenced by the lack of synergistic hemolysis (Fig. 1). Multiplex PCR was used to determine the *agr* group genotype as described previously, with appropriate control strains for *agr* groups I, II, III, and IV (11).

(iii) Antimicrobial susceptibility testing. Vancomycin, daptomycin, and linezolid MICs were measured using the microdilution Etest (AB Biodisk, Solna, Sweden; bioMérieux, Durham, NC) according to the manufacturers' instructions and following Clinical and Laboratory Standards Institute guidelines (1, 3). The VISA phenotype was defined as a vancomycin MIC of ≥4 μg/ml (33). The Etest macrodilution method was used to determine if the patient was infected with heterogeneous VISA (hVISA) as described previously (40).

(iv) Additional genotype studies. PCR was used to determine the presence of the Panton-Valentine leukocidin (PVL) and arginine catabolic mobile element (ACME) genes (6, 17). The polymorphic X region of the staphylococcal protein A (*spa*) gene was sequenced and typed according to previously described procedures (12, 31). The USA300 clone was defined as any *S. aureus* isolate that carried *spa* type motif MBQBLO and was PVL positive and ACME positive, as previously validated (14).

Clinical and outcome variable definitions. The primary outcome of interest was 30-day in-hospital mortality, defined as death occurring in the hospital during the period from culture collection to 30 days after culture collection. Severity of illness was measured 24 h before the time the culture was obtained, using the modified APS. If the blood culture was obtained within 24 h of hospital admission, the APS at the time of admission was calculated. We chose to measure severity of illness as close to exactly 24 h before culture collection as possible. This was done in order to address the underlying severity of illness before infection. Measurement of the severity of illness after culture collection would measure the severity of the bacteremia, which should not be controlled for since it is in the causal pathway (20, 29). The modified APS is based on the acute physiology and chronic health evaluation (APACHE) III score (16). Since the APACHE III was designed for use in intensive care unit (ICU) patients, the score has been modified by excluding variables that are not applicable to a more general inpatient population, as in several previously published studies (16, 22, 32). Aggregate comorbidity was measured using the chronic disease score. Medications prescribed in the first 24 h of admission were given a weighted value which was used to calculate the chronic disease score for each admission (39). The chronic disease score has been validated for use in studies of antibiotic resistance, including studies of methicillin-resistant *S. aureus* (MRSA), and is used in a similar fashion to the Charlson comorbidity index but may be more predictive (18). Blood cultures collected more than 48 h after admission were designated hospital-onset infections, and blood cultures collected within 48 h of admission were designated community-onset infections. Polymicrobial infection was defined as having more than one microorganism present in the same blood culture. Patients who received any antibiotics from the University of Maryland Medical Center within a year of the index admission were categorized as having received antibiotics in the past year.

Statistical analysis. Bivariable associations were assessed using the chi-square test or Fisher's exact test for categorical variables and the Student *t* test or the Wilcoxon rank sum test for continuous variables. Stratification was used to test for potential effect modification. Unadjusted relative risks (RRs) were calculated to assess the associations between categorical variables and 30-day in-hospital mortality. Cox proportional hazard models were fit to measure the hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between *agr* dysfunction and 30-day in-hospital mortality. Patients were censored upon hospital discharge. The proportional hazards assumption that the effect of a variable is constant over time was tested for each variable in the final model by assessing the interaction of the variable with a function of time. All variables that were significantly ($\alpha \leq 0.1$) associated with *agr* dysfunction or mortality in the bivariate analysis, as well as variables considered to be biologically important (e.g., methicillin resistance), were included in the initial (full) multivariable Cox proportional hazard model. Variables that were not significantly associated with the outcome ($\alpha > 0.05$) were removed from the full multivariable model in succession. Each of the removed variables was then reinserted into the model to assess whether it was a confounder, which was considered the case if the variable altered the regression coefficient of the primary exposure variable by >20%. If so, that variable was included in the model.

Since other studies have shown that the risk of mortality among bacteremic patients changes depending on underlying severity of illness, the cohort was divided into quartiles by initial severity-of-illness scores, and separate analyses were performed (15). To assess effect modification with severity of illness, we also examined the significance of an interaction term representing the interaction between a high severity-of-illness score and *agr* function. All analyses were performed using SAS software (SAS Institute, Cary, NC), version 9.1.

RESULTS

There were 814 episodes of *S. aureus* bacteremia in 756 unique patients who had *S. aureus* isolated from the blood during the study period. Of these, 86% had received vancomycin on the index admission and 42% had received antibiotics in

TABLE 1. Pathogen and patient characteristics stratified by *agr* function

Characteristic	No. (%) of infections with characteristic (unless indicated otherwise)			*P* value
	Strains with functional *agr* (n = 633 [77.8% of infections])	Strains with dysfunctional *agr* (n = 181 [22.2% of infections])	All strains (n = 814)	
Pathogen characteristics				
Methicillin resistance	372 (58.8)	116 (64.1)	488 (60.0)	0.20
agr group[a]				<0.01
I	363 (58.3)	60 (34.1)	423 (52.0)	
II	221 (35.4)	79 (44.9)	300 (36.8)	
III	36 (5.8)	35 (19.9)	71 (8.7)	
IV	3 (0.5)	2 (1.1)	5 (0.6)	
Vancomycin MIC (μg/ml)				<0.01
0.5	1 (0.2)	3 (1.7)	4 (0.5)	
0.75	18 (2.8)	14 (7.7)	32 (3.9)	
1	113 (17.8)	45 (24.9)	158 (19.4)	
1.5	339 (53.6)	89 (49.2)	427 (52.5)	
2	149 (23.5)	24 (13.3)	173 (21.3)	
>2	13 (2.1)	6 (3.3)	19 (2.3)	
hVISA phenotype	27 (4.3)	8 (4.4)	35 (4.3)	0.93
Hospital-onset infection	223 (35.2)	72 (39.8)	295 (36.2)	0.26
USA300 clone	171 (27.0)	19 (10.5)	190 (23.3)	<0.01
spa type motif MDMGMK	156 (24.6)	78 (43.1)	234 (28.8)	<0.01
Yr of isolation				0.01
2003	158 (25.0)	51 (28.2)	209 (25.7)	
2004	147 (23.2)	57 (31.5)	204 (25.1)	
2005	151 (23.8)	34 (18.8)	185 (22.7)	
2006	117 (18.5)	33 (18.2)	150 (18.4)	
2007[b]	60 (9.5)	6 (3.3)	66 (8.1)	
Patient characteristics				
Age (yr) (mean ± SD)	48 ± 16	52 ± 17	49 ± 16	<0.01
Female sex	240 (37.9)	70 (38.7)	310 (38.1)	0.85
Admitted to ICU prior to culture collection	186 (29.4)	61 (33.7)	247 (30.3)	0.27
Endocarditis	110 (17.4)	28 (15.5)	138 (17.0)	0.55
Median (IQR) severity-of-illness score	16 (8–28)	19 (11–28)	17 (9–28)	0.08
Median (IQR) chronic disease score	1.0 (0–2.1)	1.0 (1.0–2.2)	1.0 (0–2.1)	0.14
Received vancomycin on index admission	540 (85.3)	160 (88.4)	700 (86.0)	0.29
Receipt of antibiotics during previous yr	263 (41.6)	75 (41.4)	338 (41.5)	0.98
Injection drug use	219 (34.6)	32 (17.7)	251 (30.8)	<0.01
Presence of central venous catheter before culture collection	322 (63.0)	114 (50.9)	436 (53.6)	<0.01

[a] Fifteen isolates were unable to be typed for *agr* group.
[b] Only the first 6 months of 2007 were assessed.

the past year. One hundred nine (13.4%) patients died in the hospital within 30 days of culture collection.

Among the *S. aureus* isolates collected, 63% were considered community-onset infections (culture collection within 48 hours of admission). Sixty percent of the isolates were MRSA, 2.1% were nonsusceptible to daptomycin, and all were susceptible to linezolid. The vancomycin MIC distribution of the isolates was as follows: <1 μg/ml, 4.3%; 1 μg/ml, 19.6%; 1.5 μg/ml, 52.5%; 2 μg/ml, 21.3%; and >2 μg/ml, 2.3%. Two (0.2%) isolates were VISA, and 4.3% were hVISA. Genotyping analyses at the *agr* locus found that 52% of isolates were of *agr* group I, 37% were of *agr* group II, 9% were of *agr* group III, <1% were of *agr* group IV, and 2% were unable to be typed. Twenty-two percent of the isolates (n = 181) had *agr* dysfunction as measured by the δ-hemolysin assay.

Thirty-seven percent (n = 300) of the isolates had a *spa* type associated with the ST8 clone, and among these, 63% were classified as USA300 (i.e., MBQBLO motif positive, PVL positive, and ACME positive). Thirty percent (n = 245) of the isolates had *spa* types associated with the ST5 clone, and 5% of the isolates had *spa* types associated with the ST30 clone. More details about the *S. aureus* clones in this cohort can be found in Table S1 in the supplemental material.

S. aureus isolates with dysfunctional *agr* were more likely to carry the MDMGMK *spa* type motif and to be of *agr* group II and had lower vancomycin MICs than isolates with functional *agr* ($P < 0.05$). Patients infected with *S. aureus* with dysfunctional *agr* were more likely to have a central venous catheter before culture collection, were more likely to be admitted in an earlier year, and were less likely to be injection drug users ($P < 0.01$) than patients infected with *S. aureus* with functional *agr* (Table 1).

TABLE 2. Pathogen characteristics stratified by 30-day in-hospital mortality[a]

Characteristic	No. (%) of admissions with characteristic			*P* value
	Nonsurvivors (*n* = 109 [13.4% of admissions])	Survivors (*n* = 705 [86.6% of admissions])	All admissions (*n* = 814)	
agr dysfunction	33 (30.3)	149 (21.0)	182 (22.2)	0.03
Methicillin resistance	70 (64.2)	418 (59.3)	488 (60.0)	0.33
hVISA phenotype	7 (6.4)	28 (4.0)	35 (4.3)	0.24
Daptomycin MIC of >1 μg/ml	3 (2.9)	13 (2.0)	16 (2.1)	0.53
Vancomycin MIC (μg/ml)				0.78
0.5	1 (0.9)	3 (0.4)	4 (0.5)	
0.75	7 (6.4)	25 (3.6)	32 (3.8)	
1	19 (17.6)	140 (19.9)	159 (19.6)	
1.5	54 (50.0)	373 (52.9)	427 (52.5)	
2	26 (24.1)	147 (20.9)	173 (21.3)	
>2	2 (1.9)	17 (2.4)	19 (2.3)	
Polymicrobial infection	12 (11.0)	77 (10.9)	89 (10.9)	0.98
agr group II	50 (45.9)	250 (35.5)	300 (36.8)	0.05
PVL	27 (24.8)	224 (31.8)	251 (30.8)	0.14
ACME[b]	30 (27.5)	187 (28.4)	217 (28.2)	0.73
USA300 clone[c]	21 (19.3)	169 (24.0)	190 (23.3)	0.28

[a] No isolates were nonsusceptible to linezolid.
[b] ACME was measured for all MRSA isolates and for methicillin-susceptible *S. aureus* isolates that were PVL positive and had *spa* type motif MBQBLO.
[c] The USA300 clone was defined as MBQBLO *spa* motif positive, PVL positive, and ACME positive.

TABLE 3. Adjusted association between *agr* dysfunction and mortality, stratified by severity of illness

Quartile based on severity of illness	Mortality (%) among patients infected with *S. aureus*		Association between *agr* dysfunction and mortality (adjusted HR [95% CI])[a]
	Strains with dysfunctional *agr*	Strains with functional *agr*	
4th (modified APS of >28; *n* = 216 [27% of patients])	44.0	25.3	1.82 (1.03–3.21)
3rd (modified APS of >17 and ≤28; *n* = 195 [24% of patients])	14.8	13.5	1.01 (0.41–2.48)
2nd (modified APS of >9 and ≤17; *n* = 208 [26% of patients])	4.2	5.0	0.43 (0.08–2.47)
1st (modified APS of ≤9; *n* = 195 [24% of patients])	3.3	4.2	0.28 (0.02–3.40)

[a] Statistically adjusted for vancomycin MIC, receipt of vancomycin, aggregate comorbidity, methicillin resistance, presence of a central venous catheter, previous admission in the past year, and age, using Cox proportional hazard models.

When the bivariable statistical associations between molecular characteristics and 30-day in-hospital mortality were assessed, *agr* dysfunction was the only pathogen characteristic evaluated that was statistically associated with 30-day in-hospital mortality ($P = 0.03$). Other pathogen characteristics, including methicillin resistance, increased daptomycin, linezolid, or vancomycin MIC, hVISA phenotype, polymicrobial infection, presence of the PVL or ACME gene, and pulsed-field gel electrophoresis (PFGE) type USA300, were not associated with 30-day in-hospital mortality ($P > 0.05$) (Table 2). There was a trend toward increased mortality among patients infected with *S. aureus* with *agr* type II (unadjusted RR = 1.41; 95% CI, 0.99 to 2.00).

In total, 18% of patients infected with *S. aureus* with dysfunctional *agr* died within 30 days of culture collection, compared to 12% of patients infected with *S. aureus* with functional *agr*. Infection with *S. aureus* with dysfunctional *agr* was significantly associated with 30-day in-hospital mortality (unadjusted RR = 1.52; 95% CI, 1.05 to 2.21). In contrast, methicillin resistance (i.e., presence of the *mecA* gene) was not statistically associated with 30-day in-hospital mortality (unadjusted RR = 1.20; 95% CI, 0.83 to 1.73).

We used contingency tables to calculate the sensitivity of the δ-hemolysin assay for predicting mortality. Lack of δ-hemolysin production (i.e., *agr* dysfunction) was able to predict mortality with a sensitivity of 30%, specificity of 79%, positive predictive value of 18%, and negative predictive value of 88%. There was a nonsignificant trend toward a higher hazard of 30-day in-hospital mortality among patients infected with *S. aureus* with dysfunctional *agr* than among patients infected with *S. aureus* with functional *agr* after statistically adjusting for severity of illness, age, aggregate comorbidity, methicillin resistance, previous admission in the past year, presence of a central venous catheter, receipt of vancomycin at any time during the index admission, and vancomycin MIC (adjusted HR = 1.34; 95% CI, 0.87 to 2.06; $P = 0.18$).

Eighty-six percent (700/814 admissions) of the total cohort received vancomycin during the index admission. Ninety percent of the most severely ill patients (modified APS of >28) received vancomycin empirically or definitively. Limiting the cohort to patients who received vancomycin empirically or definitively did not change the magnitude of the adjusted association between loss of *agr* function and 30-day in-hospital mortality.

When the cohort was stratified into quartiles by severity of illness, 4.1% of patients in quartile 1 (modified APS of <9) died within 30 days of hospitalization, 4.8% of patients in quartile 2 died (modified APS of 10 to 17), 13.9% of patients in quartile 3 died (modified APS of 18 to 28), and 29.6% of patients in quartile 4 died (modified APS of ≥28). Among the patients in the quartile with the highest severity-of-illness scores, patients infected with *S. aureus* with *agr* dysfunction had a significantly higher hazard of mortality than patients infected with *S. aureus* with functional *agr* after statistically adjusting for vancomycin MIC, receipt of vancomycin, aggregate comorbidity, methicillin resistance, presence of a central venous catheter, previous admission in the past year, and age (adjusted HR = 1.82; 95% CI, 1.03 to 3.21; $P = 0.04$) (Table 3). Similarly, when we assessed the interaction between a high severity-of-illness score and *agr* dysfunction among the entire cohort, the adjusted interaction term was statistically significant ($P = 0.049$).

Subcohort analyses were also performed. When repeat admissions were excluded from the cohort, the association between infection with *S. aureus* with *agr* dysfunction and mortality remained significant (unadjusted RR = 1.50; 95% CI,

1.03 to 2.19). Another subgroup analysis was performed among 245 patients infected with *S. aureus* strains with *spa* types that correlate with the ST5 multilocus sequence type, which is considered to be made up of health care-associated strains. In this subgroup, infection with *S. aureus* with dysfunctional *agr* was a significant risk factor for mortality (unadjusted RR = 1.70; 95% CI, 1.02 to 2.82).

DISCUSSION

Although *S. aureus* bloodstream infections are associated with high mortality in general, specific pathogen and host factors have been found to be predictive of inferior responses to vancomycin therapy and of poor outcomes. This is the first study to demonstrate an independent association between *agr* dysfunction and higher 30-day mortality in severely ill patients with *S. aureus* bacteremia.

In our study, *agr* dysfunction was significantly associated with mortality only among the most severely ill patients. While *agr* dysfunction was associated with a significant (91%) increase in mortality for the sickest quartile of patients, there was no association for the other quartiles (Table 3). Based on what is known about the effects of *agr* dysfunction on *S. aureus*, we hypothesize that this observation has its foundation in two components of the host-pathogen-drug triad. First, it has been determined that *S. aureus* with a loss of or reduced *agr* function is not killed as effectively *in vitro* by vancomycin as *S. aureus* with preserved *agr* function. Pharmacodynamic models have shown a decrease in vancomycin bactericidal activity against *S. aureus* with dysfunctional *agr* compared to *S. aureus* with functional *agr*, and vancomycin kill-curve studies of clinical MRSA isolates stratified by δ-hemolysin production demonstrated significantly less killing by vancomycin of MRSA strains that did not produce δ-hemolysin than of those strains that did produce δ-hemolysin (24, 28, 36, 37). While the mechanism of this phenomenon is not understood, it is believed to depend in part on diminished autolysis of *S. aureus* with dysfunctional *agr*. A host who is severely ill may rely on bactericidal therapy in order to survive much more so than a host who is not as sick, with potentially a more intact immune system. While the importance of bactericidal therapy in the prompt eradication of bacteremia has been demonstrated, such analyses have not been done based on severity of host illness and on actual mortality. Since this was a retrospective cohort study, we did not have the means to measure duration of bacteremia but suspect that this effect on patient mortality by strains with *agr* dysfunction may lie in the longer duration of bacteremia, given the ample data linking duration of bacteremia to increased mortality and *agr* dysfunction to prolonged bacteremia (2, 7). Testing for *agr* dysfunction may identify a subgroup of patients who need close vancomycin therapeutic monitoring or should receive other antistaphylococcal antibiotics.

A second component that we believe is affected by *agr* dysfunction is the pathogenicity of the organism in bloodstream infections. *agr* dysfunction has been linked to reduced killing by innate host defense cationic peptides from platelets called thrombin-induced platelet microbicidal proteins (tPMPs). Resistance to these peptides has been associated with prolonged bacteremia and an enhanced ability to establish endovascular infection such as endocarditis (7, 25). Fowler et al. compared 21 patients with persistent MRSA bacteremia to 18 patients with resolving MRSA bacteremia and found that patients with persistent bacteremia were significantly more likely to have strains with reduced susceptibility to tPMPs and defective δ-hemolysin production than patients with resolving bacteremia. Therefore, a host with a low severity of illness may be able to overcome conditions of prolonged bacteremia and endocarditis much more effectively, thus ensuring survival, than a host with a high severity of illness.

Recently, much emphasis has been placed on the association between other elements of *S. aureus*, such as *mecA* and vancomycin MIC, and clinical outcomes. However, in our bivariable analysis, *agr* dysfunction was the only characteristic of *S. aureus* that was associated with patient mortality. Continued primary emphasis on *mecA*$^+$ *S. aureus* or on MRSA, in both the clinical and infection prevention senses, may not be an optimal strategy to improve patient outcomes and patient safety going forward. Future studies should assess the ability of *agr* function testing to predict poor patient outcomes and optimize antibiotic therapy. In addition, if these findings are validated, then infection control efforts might be directed at preventing the transmission of *S. aureus* with *agr* dysfunction instead of or in addition to strains that contain *mecA*.

Potential limitations in the clinical component of this study include its retrospective, observational nature and the fact that it reflects the experience of a single center. Thus, there is a risk of unmeasured confounding. However, a randomized controlled trial would not be ethical because patients cannot be randomized to infection with *S. aureus* with dysfunctional *agr.* In addition, as stated above, we did not quantify the duration of bacteremia, a critical determinant of patient mortality in *S. aureus* bacteremia. With respect to limitations in the laboratory phase of this study, it is possible that *agr* dysfunction mutations could have occurred in the laboratory rather than *in vivo*. However, Traber et al. demonstrated that *agr* mutations, which may lead to *agr* dysfunction, occur before or during an infection rather than during specimen handling (35). Also, posthandling mutations would be independent of patient mortality and would be expected to bias our results only toward the null hypothesis. Another laboratory limitation is that δ-hemolysin assay may not be as sensitive a marker for *agr* dysfunction as other laboratory tests, such as Northern blot hybridization to detect RNAIII production (41). When Shopsin et al. measured δ-hemolysin activity as well as performing Northern blotting for RNAIII and assessment of exoprotein profiles, they found that δ-hemolysin activity was a fairly specific marker for *agr* dysfunction (30). Measurement of δ-hemolysin activity is also easier and less expensive than Northern blotting (27).

In summary, this study demonstrates that *agr* dysfunction in *S. aureus* bacteremia isolates is associated independently with excessive mortality among severely ill patients. The δ-hemolysin assay to semiquantitatively examine *agr* function may be a simple and inexpensive approach for predicting patient outcomes and potentially optimizing antibiotic therapy.

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