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MAJOR ARTICLE

Intermittent Negative Blood Cultures in *Staphylococcus aureus* Bacteremia; a Retrospective Study of 1071 Episodes

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Background. Recommended management of *Staphylococcus aureus* bacteremia (SAB) includes follow-up blood culture sets (BCs) to determine the duration of bacteremia. Duration of bacteremia is an important prognostic factor in SAB, and follow-up BCs have a critical role in differentiation of uncomplicated and complicated SAB. However, intermittent negative BCs occur in SAB. Clinical guidelines for SAB management do not specify an approach to follow-up BCs' collection or define the number of negative BCs required to demonstrate resolution of bacteremia. This study assessed the frequency of intermittent negative BCs in SAB and used these findings to formulate a recommendation for collection of follow-up BCs.

Methods. This retrospective study reviewed 1071 episodes of SAB. Clinical and microbiological data including the duration of bacteremia and the occurrence of intermittent negative BCs (those preceded and followed by positive cultures) were considered.

Results. Intermittent bacteremia occurred in 13% (140/1071) of episodes. A single negative BC on days 1–3 had a predictive value of 87%–93% for resolution of bacteremia, although this was improved if all BCs collected within the same day were considered.

Conclusions. Intermittent negative BCs are common in SAB. Given this, we would not recommend accepting a single negative BC as demonstrating resolution of the bacteremia. This is particularly important if a patient is to be classified as having uncomplicated SAB.

Keywords. *Staphylococcus aureus*; bacteremia; blood cultures.

Staphylococcus aureus bacteremia (SAB) is both common, with an estimated incidence of 10 to 30 per 100 000 person years [1], and serious, with a 30-day mortality around 16% in the modern era [2]. Limited clinical trials in the management of SAB [3] are reflected in the variability in clinical practice [4, 5]. The management of SAB is identified as one of the key areas for ongoing clinical research in infectious diseases [3, 6–8].

Duration of bacteremia is an important prognostic factor in SAB [9–11]. SAB management guidelines recommend follow-up BCs at days 2–4 to determine the duration of bacteremia [12]. Persistent bacteremia >72 hours after initiation of effective antimicrobial therapy meets criteria for complicated SAB and indicates at least 4 weeks of intravenous antimicrobial therapy, as per consensus opinion clinical guidelines [12], although the optimal treatment duration remains to be confirmed in prospective studies. This can be contrasted with treatment durations as short as 2 weeks for patients who clear the bacteremia within 72 hours, in addition to other factors, including echocardiography not suggestive of infective endocarditis, no complicated source of bacteremia that indicates prolonged treatments, and no evidence of metastatic infection [12]. Differentiation between complicated and uncomplicated SAB is of critical importance [3] and is one of the most important roles of follow-up BCs [9–11].

Despite the importance of follow-up BCs, the timing and number of negative BCs required to demonstrate clearance of bacteremia have not been defined in the research literature or clinical guidelines [12]. The sensitivity of a single BC is ~90% during SAB [13]. However, this varies depending on the volume of blood collected, the concentration of bacteria, the presence of antimicrobials, and the media used [14]. As such, a single negative BC may not definitively conclude the period of bacteremia.

This study reviewed 1071 patients with SAB, including 140 patients with intermittent negative BCs. This analysis is used to make recommendations regarding an optimal strategy for the collection of BCs to determine resolution of bacteremia in SAB.

METHODS

This retrospective study was performed at Monash Health, a large metropolitan hospital network in Melbourne, Australia, with 2150 inpatient beds across 5 hospitals serviced by a single microbiology laboratory. All SABs are notified to the treating medical team by medical microbiology staff, and recommended management is discussed. Infectious diseases consultation is almost universal, but not mandatory. An electronic medical record for pathology orders and prescribing was not in use at the time of the study.

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The blood culture media used in the study were BACTEC PLUS Aerobic/F, BACTEC Lytic/10 Anaerobic/F, and BACTEC Peds Plus/F in the BACTEC FX incubator (Becton Dickinson Instrument Systems, Sparks, MD, USA), all with routine 5-day incubation at 35°C and automated detection of positivity. A BC was defined as either 1 or 2 blood culture bottles collected during a single peripheral venepuncture or vascular access aspiration. A set was considered positive if either or both bottles subsequently isolated *S. aureus*.

We identified all episodes of SAB with positive BCs collected in our health network in adults and pediatric patients occurring between 2013 and October 2018 using the laboratory information system (LIS). Each episode of SAB was defined by a BCs that recovered *S. aureus* without SAB in the preceding 14 days. The first day of positive *S. aureus* BCs was defined as day 0. BCs were considered intermittently negative if a positive BC both preceded and followed a negative BC within the first 14 days of the episode; this included BCs collected at separate times within the same calendar day. We included an additional analysis in which BCs were considered intermittently negative only when all sets collected within a calendar day were negative. Recurrent episodes of SAB were defined as *S. aureus*–positive BCs >14 days after onset of the preceding episode; we did not distinguish between recrudescence and re-infection. Median follow-up was 1056 days, with a range from 30 to 2161 days. The LIS and medical records were used to collect epidemiological data, clinical diagnosis, and treatment summaries.

Data were de-identified and recorded in a secure electronic database (Excel). Statistical analysis was performed using statistical software (Stata 13). Categorical values were analyzed by chi-square test, continuous values were analyzed using the Student *t* test, and nonparametric continuous values were analyzed by the Mann-Whitney *U* test. This retrospective quality assurance study received a quality and service improvement waiver (RES-18-0000-775Q) from the Monash Health Human Research Ethics Committee.

RESULTS

S. aureus was isolated from 2734 (1.3%) of a total of 202 924 BCs, which included 2510 sets with paired aerobic and anaerobic bottles: 25 with aerobic bottles only, 22 with anaerobic bottles only, and 177 with pediatric bottles only. Table 1 summarizes the epidemiology of 1071 episodes of SAB in 1022 individual patients. Within this cohort, there were 140 episodes of intermittent bacteremia, representing 136 individuals. The age and sex of individuals with intermittent bacteremia were similar to those of the general cohort (Table 1). However, individuals with intermittent bacteremia were more likely to have a longer duration of bacteremia, methicillin-resistant *S. aureus* (MRSA), and to have recurrent SAB (Table 1).

Table 2 describes initial and follow-up BC results for 1071 patients with SAB. In total, there were 257 negative BCs that represented intermittent negative BCs; these were most common on days 1–3, with 142 intermittent negative BCs representing 95 different episodes. We considered the negative predictive value (NPV) of a negative BC on each day to exclude intermittent bacteremia. This was lowest on day 1, at 87%, and increased to ≥95% from day 4 onward. The frequency of intermittent negative BCs reduced if we defined intermittent negativity as occurring only when all BCs collected within a single day, even if collected as different sets and at different times, were negative. If this definition was applied, there would have been 56 fewer episodes with intermittent negative BCs, and the NPV increased to 91% and 93% on days 1 and 2 before remaining above 95% (Table 2). When we compared these 2 definitions of intermittent negative BCs, then on occasions in which multiple BCs were collected within a day, the majority, 71/86 (83%), were redefined to no longer represent intermittent negative BCs.

For those BCs containing an anaerobic and aerobic bottle, the proportion in which both bottles recovered *S. aureus* decreased from 62% on day 1 to 35% from day 7 onwards (Table 3). The time to automated detection of BC positivity also increased; 86% of positive BCs were identified after ≤24 hours' incubation on day 1 of the period of bacteremia. The proportion of BCs flagging positive within this incubation period fell to 67% by day 7 for the subset who remained bacteremic (Table 3). However, ≥95% of cultures still flagged as positive after 2 days of incubation throughout this period (Table 3).

Table 1. Epidemiology of Episodes With Continuous Positive or Intermittent Negative Blood Culture Sets in *Staphylococcus aureus* Bacteremia

	All	Continuous Positive BCs	Intermittent Negative BCs	*P*
Total episodes, No.	1077	938	140	
Male, No.	672	585	87	.96[a]
Age, mean, y	57.7	58.0	55.7	.31[b]
Duration, median days (IQR)	2 (2)	1 (2)	5 (5)	<.001[c]
MRSA, No. (%)	188 (17.5)	155 (16.5)	33 (23.6)	.04[a]
Recurrent SAB, No. (%)	43	31 (3)	12 (9)	.003[a]

Abbreviations: BCs, blood culture sets; IQR, interquartile range; MRSA, methicillin-resistant *S. aureus*; SAB, *S. aureus* bacteremia.

[a]Chi-square test.

[b]Student *t* test.

[c]Mann-Whitney *U* test.

Table 2. Initial and Follow-up Blood Culture Set Results for 1071 Patients With *Staphylococcus aureus* Bacteremia

Day	No. BCs Collected	Episodes With BCs Collected	Episodes With Single BCs Collected	Episodes With Positive BCs	Episodes With Intermittent Negative BCs	Episodes With Intermittent Negative BCs (Considering all BCs Collected Within Day)	Predictive Value of Single Negative BCs[a]	Predictive Value of Negative BCs (Considering all BCs Collected Within Day)[a]
1	964	650	409	419	38	23	0.87	0.91
2	792	649	551	276	37	26	0.91	0.93
3	759	660	588	185	35	27	0.93	0.95
4	660	594	540	132	22	18	0.95	0.96
5	542	492	447	83	20	18	0.95	0.96
6	465	405	359	56	18	13	0.94	0.96
7	348	304	266	47	14	8	0.95	0.97
8	300	252	210	26	11	9	0.96	0.96
9	245	212	186	22	3	3	0.99	0.98
10	175	149	126	13	3	2	0.98	0.99
11	186	152	126	9	4	4	0.97	0.97
12	151	123	101	7	4	3	0.97	0.97
13	127	105	84	10	0	0	1	1
14	103	83	69	4	3	2	0.97	0.95

Abbreviation: BCs, blood culture sets.

[a]Probability that a negative BC is not an intermittent negative.

Table 3. Positive Blood Culture Set Incubation Periods and Concordance of Paired Bottles

Days From Index BCs	No. BCs With Paired Anaerobic & Aerobic Bottles—Pos BCs/BCs Collected (%)	Both Bottles Positive[a] (%)	Positive Within Incubation Period (%)	
			Day 1	Day 2
1	578/881 (66)	62	86	97
2	305/728 (42)	52	79	97
3	207/701 (30)	49	75	96
4	146/609 (24)	49	78	95
5	94/512 (18)	37	82	98
6	64/433 (15)	41	82	98
7	52/328 (16)	35	67	96
>7	102/1193 (09)	34	69	95

Abbreviation: BCs, blood culture sets.

[a]For those blood culture sets with both an aerobic and anaerobic bottle collected.

Consecutive negative intermittent negative BCs, defined as intermittent negative BCs occurring on ≥2 consecutive days, occurred for 44 patients (4%), representing 32% of the cohort with any intermittent bacteremia. Episodes of early consecutive intermittent negative BCs were also noted; 9 patients had intermittent negative BCs collected on days 1 and 2, of which a further 4 patients had intermittent negative BCs on day 3 before subsequent positive BCs. Of the 4 patients with intermittent negative BCs on days 1–3, 2 had clinically recognized endocarditis and another had metastatic infection with septic pulmonary emboli and so would not have been misclassified as uncomplicated bacteremia. When we used the alternate intermittent negative BC definition and considered all BCs collected on the same day, we identified 35 patients with consecutive negative BCs, with 5 patients having intermittent negatives on days 1 and 2 and 3 patients having consecutive intermittent negative BCs on days 1–3.

We assessed for potential misclassification of patients as having an uncomplicated SAB if no further BCs were collected after the first intermittent negative BCs. There were 55 episodes with an intermittent negative BC between days 1 and 3, with a final diagnosis of either intravenous catheter–associated infection, SAB secondary to skin and soft tissue infection, or SAB without identified source and no metastatic infection identified.

DISCUSSION

These results demonstrate that intermittent negative BCs, including consecutive intermittent negative BCs, are common in SAB and on day 1, 2, or 3, a single negative BC had an NPV of 87%, 91%, and 93%, respectively (Table 2). Although the

apparent NPV of a single blood culture improves after day 3, this is largely due to reduced pretest probability, as the proportion of cases with ongoing bacteremia is reduced. An early intermittent negative BC could, in conjunction with other clinical factors, lead to a patient being misclassified as uncomplicated SAB and receiving inadequate therapy. The risk of this is reduced, but not eliminated, when considering all BCs collected on a calendar day. In this study, there were 55 episodes, 5% of the total SAB cohort, in which cases could have been misclassified as uncomplicated due to intermittent negative BCs if no further BCs were collected after the first negative BC. Our recommendation for multiple negative BCs is particularly important in the setting of MRSA and recurrent SAB, both of which are associated with increased rates of intermittent negative blood BCs. We were unable to determine if the association of intermittent bacteremia and recurrent SAB may be a consequence of SAB misclassification and subsequent treatment failure.

This study has limitations, which are largely inherent to its retrospective design. We have likely underestimated the prevalence of intermittent negative BCs given that there is no established protocol for collection of clearance BCs at our institution and some intermittent SAB would have been incorrectly classified as having resolved after a single negative BC. The reason for intermittent negative BCs was not determined in this study. The most important factor affecting BC sensitivity is volume of collection [15, 16]. The concentration of colony-forming units per milliliter (CFU/mL) of *S. aureus* during periods of bacteremia varies from <1 CFU/mL to >500 CFU/mL, with endovascular infections tending toward higher concentrations [17]. Consistent with a falling CFU/mL, there was increased time to blood culture positivity and a reduction in paired positive bottles for BCs containing both aerobic and anaerobic bottles as the duration of the bacteremia extended (Table 3). We also noted that the frequency of intermittent negative BCs was reduced if we considered all BCs collected within a single calendar day (Table 2). It is unclear if this is due to improved sensitivity secondary to increased volumes collected or due to true intermittent bacteremia and different times of collection. In some cases, bacteremia is intermittent, particularly in undrained closed-space infections, including abscesses, cholangitis, and focal infections, including pneumonia, osteomyelitis, and spondylodiscitis [18]. BC sensitivity may be reduced if taken immediately after antimicrobial administration, although this effect is partially mitigated by the antimicrobial resin in BACTEC blood culture media, which significantly increases detection yield and reduces time to positivity [19]. Delays in processing BCs before incubation can result in a several-log reduction in sensitivity for automated detection of positivity [20]. The review of the medical record was limited to determining the final diagnosis. We did not attempt to discriminate between BC collection for surveillance and that for ongoing clinical concern, as this could not be routinely determined from the record. Despite these limitations, the aims of this study were to provide safe recommendations for clearance BCs that could be applied by the treating clinicians in routine clinical practice.

Management of SAB is complicated. Clinical guidelines provide some support, and adherence to these guidelines has been shown to improve outcomes; additionally, multiple studies have demonstrated improved clinical outcomes with bedside infectious diseases consultation [21, 22]. However, 87% of SAB cases are admitted by non–infectious disease home teams [23], and access to infectious diseases consulting services is not universally available. Given that early management of SAB cases is often by nonspecialist units, it is important to provide as much clarity as possible in guidelines. These findings help provide recommendations regarding the appropriate collection of clearance BCs in SAB. There is potential for follow-up BC collection strategies in SAB to be included in electronic medical record order sets. As such, we recommend that a single negative blood culture should never be accepted as sufficient evidence that bacteremia has resolved. This is particularly important if a patient is to be classified as having an uncomplicated SAB.

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