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MAJOR ARTICLE

Association of Repeated Blood Cultures With Mortality in Adult Patients With Gram-Negative Bacilli Bacteremia: A Systematic Review and Meta-analysis

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Background. Performing repeat blood cultures after an initial positive culture (ie, follow-up blood cultures [FUBCs]) in patients with gram-negative bacilli (GNB) bacteremia is controversial. We aimed to comprehensively review the association of FUBCs with improvement in patient-relevant clinical outcomes in GNB bacteremia.

Methods. We performed a systematic review and random-effects meta-analysis to calculate summary effect estimates. We used hazard ratios as the effect measure. The primary outcome was 30-day or in-hospital mortality, and secondary outcomes were length of treatment and length of hospital stay. We searched PubMed, Embase, and Cochrane Central Register of Controlled Trials (Central) without language restrictions from inception to April 29, 2022. Original clinical studies evaluating the association between FUBCs and mortality in adult patients with GNB bacteremia were included. FUBC details were reviewed. Two independent reviewers used the Risk of Bias in Non-randomised Studies of Interventions tool.

Results. We identified 9 eligible retrospective studies. In total, 7778 hospitalized patients with GNB bacteremia were included. The studies were clinically heterogeneous and had a critical risk of bias. The utilization of FUBCs varied across studies (18%–89%). Random-effects meta-analysis of covariate-adjusted estimates found that FUBC use was associated with reduced mortality. Although not a result of the meta-analysis, lengths of treatment and hospital stay were longer for patients with FUBCs than for those without. Adverse events were not reported.

Conclusions. FUBC acquisition was associated with lower mortality and longer hospital stay and treatment duration in GNB bacteremia. The risk of bias was critical, and no firm data were available to support mechanisms.

Keywords. repeat blood cultures; systematic review and meta-analysis; follow-up blood cultures; gram-negative bacilli bacteremia.

Gram-negative bacilli (GNB) bacteremia is a common and potentially fatal infection [1]. Despite receiving effective antimicrobial therapies, 20%–40% of patients die from GNB bacteremia [2, 3]. GNB bacteremia is a diverse clinical syndrome, ranging from easily treatable urinary tract infections to endovascular infections, which tend to cause persistent bacteremia. Similarly, the causative microorganisms range from pan-susceptible *Escherichia coli*, for which bacteremia clearance is easy, to difficult-to-treat *Klebsiella pneumoniae* carbapenemase (KPC)–producing organisms and multidrug-resistant *Pseudomonas aeruginosa*. Therefore, a uniform approach to management carries with it the risk of both under- and overtreatment regarding the duration of therapy. Undertreatment is associated with a worse prognosis, while overtreatment results in unnecessary costs, prolonged treatment, and longer in-hospital stays.

Follow-up blood cultures (FUBCs), that is, repeated blood cultures performed after an initial positive culture, are common tests in the management of patients with bacteremia in specific clinical scenarios [4, 5]. By repeating blood cultures, clinicians can assess the persistence of bacteremia and determine the appropriateness and duration of ongoing antimicrobial therapy. When treating *Staphylococcus aureus* bacteremia or candidemia, FUBCs constitute part of standard management [4, 5]. However, routine FUBC use in patients with GNB bacteremia is controversial [6], as some observational studies have reported an association between this practice and lower mortality risk [7–9], while others have shown an association with longer duration of hospital stay and treatment [10, 11].

The associations of specific patient and test characteristics with FUBC positivity rates have recently been extensively discussed [6, 12, 13]. We performed a formal systematic review

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and meta-analysis of available clinical data to examine the association between FUBC acquisition and clinical outcomes in patients with GNB bacteremia.

METHODS

We followed the Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines [14]. The protocol was registered with PROSPERO (registration number: CRD42020183357). Ethics review and patient consent are not required for systematic reviews or meta-analyses.

Information Sources and Search Strategies

We searched the PubMed, Embase, and Cochrane Central Register of Controlled Trials (Central) databases without language restrictions from inception to April 29, 2022, using terms including "bacteremia," "blood culture," "gram-negative," and their synonyms. The exact search strategy is available in the Supplementary Data. We also conducted monthly literature surveys using the PubMed database and Google search engine until June 29, 2022. The free text terms used in these surveys included "repeat blood cultures" or "follow-up blood cultures" crossed with "gram-negative bacteremia."

Selection Process

Two independent reviewers (J.S. and S.H. or T.T.) separately screened the titles and abstracts and examined the full-text reports of all potentially eligible articles. The reviewers resolved disagreements through discussion.

Eligibility Criteria

We included studies that evaluated the relationship between FUBC acquisition and mortality in a minimum of 10 adult patients (aged ≥18 years) with GNB bacteremia. We excluded studies that exclusively included an a priori-defined specific population, that is, patients (1) aged <18 years; (2) with neutropenia due to chemotherapy or hematopoietic stem cell transplant pretreatment; or (3) with HIV infection. However, we accepted studies if ineligible participants accounted for <10% of the total patient sample. We also excluded case reports or case series (defined as studies with <10 patients), editorials, comments, letters, review articles, and studies without extractable outcome data. Although we planned to include randomized controlled trials (RCTs) comparing clinical management with and without FUBCs, no such RCTs were eligible.

Data Collection Process

One reviewer (J.S.) extracted the descriptive data, and another (H.S. or T.T.) verified the extracted data. Two independent reviewers (J.S. and S.H.) separately extracted the quantitative data and resolved all disagreements through discussion. We contacted the authors of the primary studies via e-mail when additional data were necessary. If no response was received after multiple attempts made at least 2 weeks apart, we considered the request rejected.

Data Items

We extracted study, participant, and intervention characteristics. Study characteristics included study identification (year of publication and first author), location (country), period (enrollment years), design (retrospective vs prospective), number of centers, inclusion criteria, and exclusion criteria. Participant characteristics included the number of participants, age, sex, intensive care unit (ICU) admission, effective empirical therapy, treatment duration, and clinical outcome (28-day, 30-day, or in-hospital mortality, length of hospital stay, duration of treatment, and adverse events directly attributable to FUBCs). Intervention characteristics included the timing and proportion of acquired FUBCs, FUBC results, and their derived additional cointerventions.

Outcomes and Prioritization

Our primary outcome was all-cause mortality, which included deaths from any cause within 28 or 30 days from the onset of infection or deaths observed during hospitalization, as defined by individual studies. The secondary outcomes included the length of hospital stay, duration of treatment, and adverse events directly associated with FUBCs.

Assessment of Risk of Bias

To assess the risk of bias, we used the Risk of Bias in Non-randomized Studies of Interventions tool [15]. Two independent reviewers (J.S. and T.T.) double-rated confounders, participant selection, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selective reporting, and then determined the overall risk of bias. The reviewers resolved disagreements through discussion.

Statistical Analysis

We used hazard ratios (HRs) as the effect measure. We extracted study-level adjusted HRs estimated using a study-specified multivariable model, which typically accounted for the largest number of covariates, and their 95% confidence intervals (CIs) from each report [7–9, 16]. In cases wherein adjusted odds ratios (ORs) were reported, they were converted to adjusted HRs using a conversion formula [10]. For studies in which study-level adjusted HRs or ORs were not extractable, we performed propensity score–based logistic regression of available covariates (specifically, potential confounders of mortality) to obtain adjusted HRs using individual-level patient data (IPD) provided by the study authors [17, 18], with a metric conversion using the Perneger method [17]; only binary-type IPD were available in all such cases. One study did not exclude patients who died early [18]; thus, we excluded 14 patients who died within 2 days after

the initial positive blood culture from the IPD based on other studies.

To assess the stability of the results, the sensitivity analysis included additional studies in which adjusted HRs were not reported and IPD could not be obtained to estimate adjusted results [11, 19]. In these studies, incidence rate ratios were used instead of HRs [20].

We qualitatively investigated the clinical heterogeneity by perusing the study, participant, and intervention characteristics. Given the observed clinical heterogeneity, we performed a random-effects meta-analysis using the Sidik–Jonkman estimator for the heterogeneity standard deviation parameter, tau, the Hartung–Knapp–Sidik–Jonkman method for its 95% CI, and the Higgins–Thompson–Spiegelhalter method for its 95% prediction interval (PI) [21, 22]. We assessed statistical heterogeneity using the tau and I^2 statistics and PIs of the effect size [23, 24].

Additional Analysis

Funnel plot asymmetry was not assessed because there were <10 studies [25]. To assess result stability, we used 2 additional recommended estimators for tau in the sensitivity analysis: restricted maximum likelihood and Paule–Mandel estimators [26]. An analysis including studies with unadjusted HRs was also conducted.

Statistical Software

We used Stata 17.0 (StataCorp; College Station, TX, USA) and R software, version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set at $P < .05$.

RESULTS

Study Flow and Eligible Studies

We screened 18 223 abstracts and evaluated 43 full-text articles (Figure 1). After excluding 34 studies, we included 9 studies. The details of the excluded studies are shown in the Supplementary Data.

Study Characteristics

Nine studies (7 from North America [7, 8, 10, 11, 16, 18, 19] and 1 each from Italy [9] and South Korea [17]) included 7778 patients; the median number of included patients per study was 766 (range, 159–1702). All studies were published after 2020 (1 in 2022 [10], 3 in 2021 [7, 16, 19], and 4 in 2020 [8, 9, 11, 17]), except for 1 published in 2016 [18]. Six studies were conducted at university hospitals, 2 in a community hospital, and 1 was unspecified. All studies had a retrospective, observational design and assessed the impact of the utilization of FUBCs based on routinely collected data, including medical records derived from clinical practice, and included clinically heterogeneous patient populations in diverse clinical contexts (Table 1). Six studies included patients with GNB bacteremia only [7–11, 17], 1 study assessed both gram-positive and gram-negative bacteremia patients [18], 1 study exclusively included patients with bacteremia due to *E. coli* or *Klebsiella* spp. [19], and 1 study exclusively included patients with *Pseudomonas aeruginosa* bacteremia [16] (Table 1). Only 1 study exclusively assessed FUBCs in a relatively similar context of community-acquired infections [7], whereas other studies jointly assessed both community- and hospital-acquired infections or did not report the clinical context. In 6 studies reporting the type of unit where the patients received care, the ICU admission rates varied substantially (4%–45%) [8, 9, 10, 11, 16, 18] (Supplementary Table 1) .

Patient Characteristics

One study included 3 patients (0.3%) aged <18 years [18]. The reported distribution of comorbid conditions varied substantially for end-stage renal disease on hemodialysis (3%–20%) [7, 8, 10, 16, 17], intravascular device (15%–59%) [7, 10, 16, 17] (Supplementary Table 2), overall immunosuppressive conditions (2%–57%) [7–10, 16, 17], HIV infection (2%–5%) [8, 10], and neutropenia (2%–9%) [10, 17] (Supplementary Table 3).

Source and Etiology of Bloodstream Infection

Seven studies reported the bloodstream infection source [7–10, 16, 17, 19]; the most common was urinary tract infections (range, 11%–60%), followed by undocumented source of infections (range, 4%–25%) and intra-abdominal infections (range, 7%–18%) (Supplementary Table 4). Among the 6 studies reporting breakdown of the causative pathogens, *E. coli* and *Klebsiella* spp. were the 2 most common (joint range, 17%–60%), whereas <10% (range, 1%–9%) of patients were reported to have nonfermentative GNB (eg, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*) bacteremia [7–10, 17, 18]. Data on drug-resistant bacteria were available from 3 studies; the abundance of extended-spectrum β-lactamase– and AmpC β-lactamase–producing bacteria ranged from 19% to 23% and from 8% to 18%, respectively [17, 18, 19] (Supplementary Table 5).

Intervention Characteristics

The proportion of positive FUBCs varied across the studies (range, 3%–38%). The etiology of positive FUBCs differed from that of the initial positive culture results in 1%–6% of cases and was deemed contamination in up to 4% of cases (Table 3).

Co-intervention Characteristics

The proportion of effective empiric therapy was similar in patients with FUBCs and those without (61%–93% vs 70%–93%) [7–9]. Source control was more common in patients with

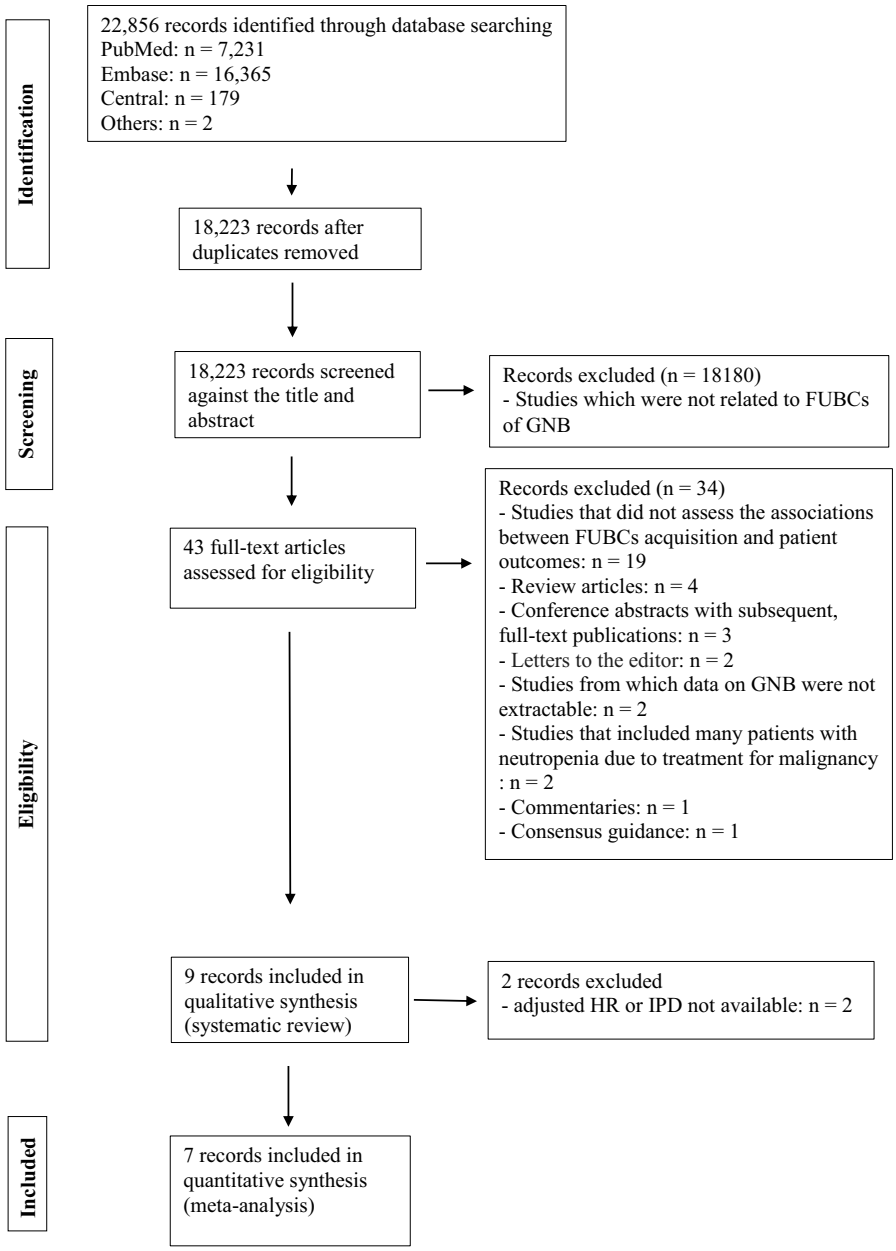

Figure 1. PRISMA flow diagram. Abbreviations: FUBCs, follow-up blood cultures; GNB, gram-negative bacilli; HR, hazard ratio; IPD, individual patient data; PRISMA, Preferred Reporting Items for Systematic Review and Meta-Analyses.

FUBCs than in those without (34%–36% vs 21%–26%) [9, 10] (Supplementary Table 6). No data were available to indicate whether antibiotics were changed, and the treatment duration was prolonged by FUBCs.

Assessment of Bias Risk

All the studies were rated as having a critical risk of bias. The risk of bias was deemed severe, particularly for confounders, participant selection, intervention classification, and missing data (Supplementary Tables 7–13, Supplementary Figure 1).

Outcomes

Mortality

Mortality was reported as 28-day and 30-day mortality in 1 [7] and 4 studies [9, 11, 16, 18], respectively, and as in-hospital mortality in 4 studies [8, 10, 17, 19]. Four studies provided adjusted HRs [7–9, 16], and 1 study provided adjusted ORs [10], which were converted to HRs using a conversion formula. We used post hoc estimated adjusted HRs in 2 additional studies [17, 18]. Statistical models and covariates accounted for the statistical corrections that varied substantially across studies

Table 1. Study Characteristics

Author	Country	Study Period	Type of Centers (No.)	Study Design	Timing of FUBCs	Reasons for FUBC Acquisition	Participants	Risk of Bias	Cotreatments	Study Outcome
Amipara et al. [7]	USA	January 2010–June 2015	Community teaching hospital (2)	Retrospective cohort study	1–4 d after initial BCs	ND	Adult patients with GN-BSI[a]	Critical	Indwelling urinary/central venous catheter	28-d mortality
Chan et al. [19]	USA	July 2014–August 2019	University-affiliated hospital (1)	Retrospective cohort study	ND	ND	Adult patients with *E. coli* or *Klebsiella* spp. BSI[b]	Critical	ND	In-hospital mortality
Elamin et al. [11]	USA	January 2017–December 2018	ND	Retrospective cohort study	ND	ND	Adult patients with GN-BSI	Critical	ND	30-d mortality
Giannella et al. [9]	Italy	January 2013–December 2016	University hospital (1)	Retrospective cohort study	1–7 d after initial BCs	ND	Adult patients with GN-BSI[a]	Critical	Source control, infectious diseases consultation	30-d mortality
Green et al. [16]	USA	November 2015–January 2020	University hospital (1)	Retrospective cohort study	1–7 d after initial BCs	Ensure clearance 48%, antibiotic therapy guide 27%, repeat until negative 9%, respond to fever 7%, others 9%[c]	Adult patients with *P. aeruginosa* bacteremia[d]	Critical	Central line removal, infectious diseases consultation	30-d mortality
Jung et al. [17]	Republic of Korea	December 2015–December 2017	University hospital (1)	Retrospective cohort study	2–7 d after initial BCs	ND	Adult patients with GN-BSI	Critical	Source control	In-hospital mortality
Maskarinec et al. [8]	USA	January 2002–June 2015	University hospital (1), university-affiliated hospital (1)	Retrospective cohort study[e]	1–7 d after initial BCs	ND	Adult patients with GN-BSI[d]	Critical	ND	In-hospital mortality
Mitaka et al. [10]	USA	January 2017–December 2018	Acute care hospital (4)	Retrospective cohort study	1–7 d after initial BCs	ND	Adult patients with GN-BSI[f]	Critical	Source control	In-hospital mortality
Wiggers et al. [18]	Canada	April 2010–June 2014	University-affiliated hospital (1)	Retrospective cohort study	2–7 d after initial BCs	ND	Adult patients with BSI[a]	Critical	ND	30-d mortality

Abbreviations: BC, blood culture; BSI, bloodstream infection; FUBC, follow-up blood culture; GN, gram-negative; ND, no date.

[a]Only first episodes were included, and recurrent episodes were excluded.

[b]Ten patients had multiple hospital admissions. This study used episodes and patients interchangeably.

[c]Reasons for FUBC acquisition were reported in 58 of the 127 patients.

[d]Only the first hospitalization was included in patients with multiple hospitalizations.

[e]Observational study of prospectively enrolled patients; the study question was posed retrospectively.

[f]Patients were included only once during each hospitalization.

(Supplementary Table 14). The median mortality rates (range) were 9% (3%–15%) in the FUBC group and 11% (3%–50%) in the no-FUBC group; the random-effects model meta-analysis suggested that FUBC use was associated with a lower mortality risk (average HR, 0.54; 95% CI, 0.42–0.69; $P < .001$); however, although the direction of effects was consistent, the range of predicted effects was wide (95% PI, 0.23–1.24; $\text{tau}^2 = 0.10$; $I^2 = 0\%$) (Figure 2).

Treatment Duration and Length of Hospital Stay

Four [8–11] and 5 [9, 10, 11, 18, 19] studies compared treatment duration and length of hospital stay between the 2 groups. Although the reported durations were longer in the FUBC group than in the no-FUBC group for treatment (range, 8–15 days vs 6–13 days, respectively; median difference, 2–5) and hospital stay (range, 7–24 days vs 4–11 days, respectively; median difference, 2–14) (Table 2), unavailability of the adjusted data precluded a formal meta-analysis. Although 1 study reported increased costs in the FUBC group [18], no adverse events directly associated with FUBCs were reported in any study.

Sensitivity Analyses

The results were similar in sensitivity analyses where different estimators of tau were used or where studies with unadjusted HRs were also included (Supplementary Table 15).

DISCUSSION

We systematically reviewed 9 retrospective studies that collectively enrolled 7778 clinically heterogeneous patients with GNB bacteremia treated individually in clinical practice. Additionally, we quantitatively synthesized the association

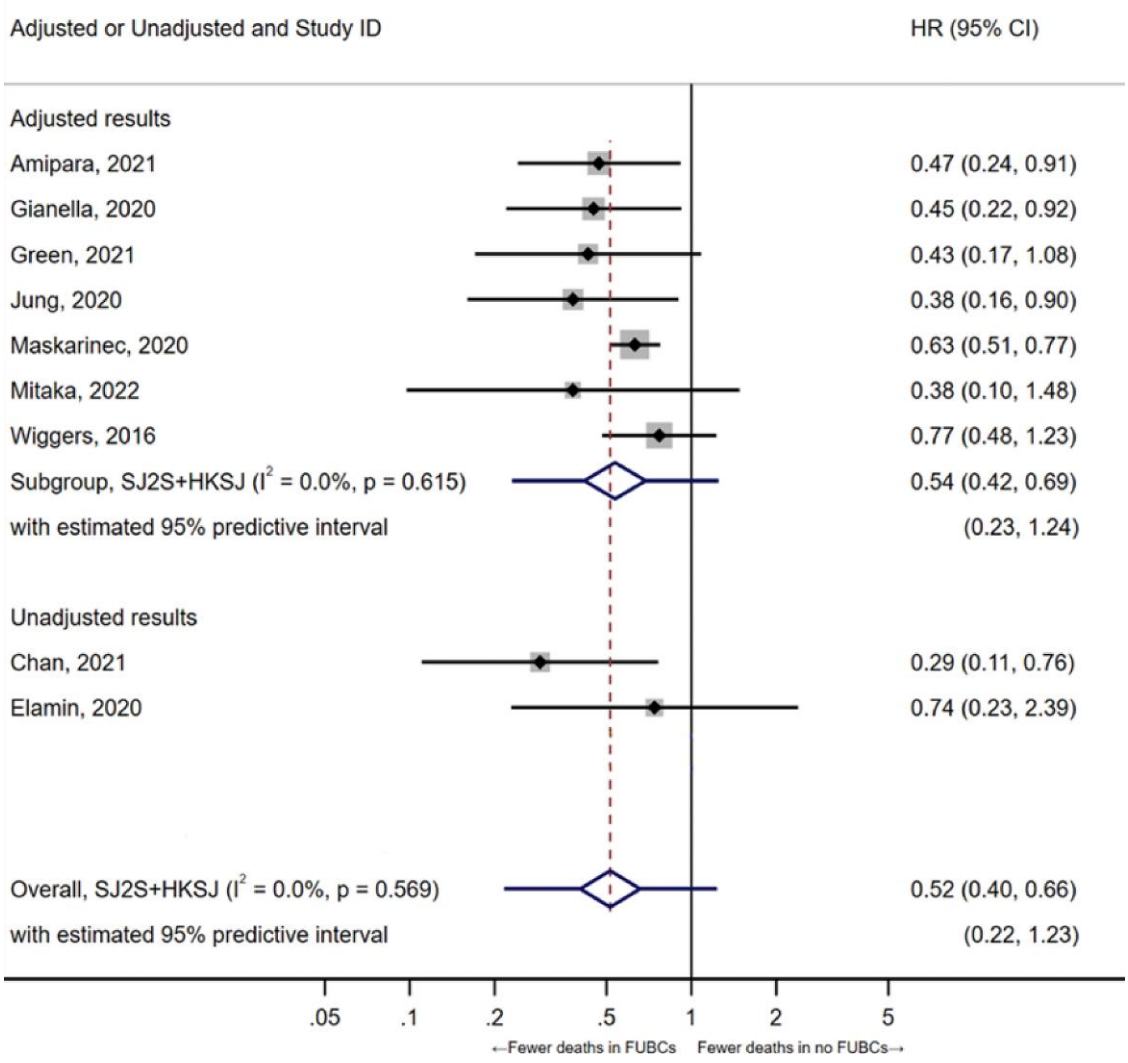

Figure 2. Forest plot of the association between the acquisition of FUBC samples and mortality. Abbreviations: CI, confidence interval; FUBC, follow-up blood culture; HKSJ, Hartung–Knapp–Sidik–Jonkman; HR, hazard ratio; ID, identification; SJ2S, Sidik–Jonkman 2-step estimator.

Table 2. Patient Characteristics

Author	Intervention Group	Patient, No. (%)	Mean or Median Age (±SD or IQR), y	Men, No. (%)	ICU Admission, No. (%)	Transplant Recipients, No. (%)	Immunosuppressant User, No. (%)	ESRD on Hemodialysis, No. (%)	Intravascular Device, No. (%)	Effective Empirical Therapy, No. (%)	Days of Treatment, Median (IQR)	Days of Hospital Stay, Median (IQR)	Mortality, No. (%)
Amipara et al. [7]	All patients	766 (100)	67 (55–79)	328 (43)	ND	ND	ND	77 (10)[a]	116 (15)[b]	710 (93)	ND	ND	69 (9)
	FUBC	219 (29)	65 (52–75)	96 (44)	ND	ND	ND	55 (25)[a]	60 (27)[b]	204 (93)	ND	ND	12 (5)
	No FUBC	547 (71)	68 (56–79)	232 (42)	ND	ND	ND	22 (4)[a]	56 (10)[b]	506 (93)	ND	ND	57 (10)
Chan et al. [19]	All patients	335 (100)	ND	186 (56)	ND	ND	ND	ND	ND	ND	ND	ND	32 (10)
	FUBC	299 (89)	60 (48–71)	174 (58)	ND	ND	ND	ND	ND	ND	ND	8 (5–18)	27 (9)
	No FUBC	36 (11)	64 (53–72)	12 (33)	ND	ND	ND	ND	ND	ND	ND	4 (3–9)	5 (14)
Elamin et al. [11]	All patients	482 (100)	ND	ND	174 (36)	ND	ND	ND	ND	ND	ND	ND	13 (3)
	FUBC	321 (67)	ND	ND	133 (41)	ND	ND	ND	ND	ND	14 (10–14)	7 (5–11)	9 (3)
	No FUBC	161 (33)	ND	ND	41 (26)	ND	ND	ND	ND	ND	11 (10–14)	5 (4–7)	4 (3)
Giannella et al. [9]	All patients	1576 (100)	69 (17)	878 (56)	126 (8)	ND	332 (21)[c]	ND	ND	1075 (68)	11 (7–15)	12 (7–22)	164 (10)
	FUBC	278 (18)	62 (16)	171 (62)	53 (19)	ND	89 (32)[c]	ND	ND	170 (61)	15 (10–21)	21 (13–36)	38 (14)
	No FUBC	1298 (82)	70 (16)	707 (55)	73 (6)	ND	243 (19)[c]	ND	ND	905 (70)	10 (7–14)	11 (7–19)	126 (10)
Green et al. [16]	All patients	159 (100)	57 (16)	94 (59)	73 (45)	ND	ND	31 (20)	94 (59)[b]	ND	ND	ND	40 (25)
	FUBC	127 (80)	57 (17)	76 (60)	60 (46)	ND	ND	23 (18)	77 (61)[b]	ND	ND	ND	24 (19)
	No FUBC	32 (20)	59 (15)	18 (56)	13 (41)	ND	ND	8 (25)	17 (53)[b]	ND	ND	ND	16 (50)
Jung et al. [17]	All patients	1481 (100)	69 (14)	745 (50)	ND	68 (5)[d]	92 (6)[e]	43 (3)[f]	327 (22)	ND	ND	ND	105 (7)
	FUBC	1276 (86)	69 (14)	628 (49)	ND	62 (5)[d]	89 (7)[e]	41 (3)[f]	308 (24)	1050 (82)	ND	ND	87 (7)
	No FUBC	205 (14)	69 (14)	117 (57)	ND	6 (3)[d]	3 (1)[e]	2 (1)[f]	19 (9)	ND	ND	ND	18 (9)
Maskarinec et al. [8]	All patients	1702 (100)	61 (ND)	936 (55)	64 (4)	232 (14)[g]	411 (24)[e]	172 (10)	ND	1075 (63)[h]	ND	ND	284 (17)[i]
	FUBC	1164 (68)	60 (16)	632 (54)	41 (4)	188 (16)[g]	305 (26)[e]	135 (12)	ND	729 (63)[h]	15 (13–19)	ND	176 (15)[ii]
	No FUBC	538 (32)	62 (16)	304 (57)	23 (4)	44 (8)[g]	106 (20)[e]	37 (7)	ND	346 (64)[h]	13 (8–16)	ND	108 (20)[i]
Mitaka et al. [10]	All patients	376 (100)	ND	174 (46)	138 (37)	ND	ND	43 (11)	115 (31)	ND	ND	ND	ND
	FUBC	271 (72)	69 (59–82)	123 (45)	110 (40)	ND	ND	37 (14)	93 (34)	ND	8 (5.5–13)[j]	9 (6–14)[j]	4 (5)[j]
	No FUBC	105 (28)	73 (64–83)	51 (49)	28 (27)	ND	ND	6 (6)	22 (21)	ND	6 (4–10)[j]	7 (4.5–10.5)[ij]	10 (11)[ij]

Table 2. Continued

Author	Intervention Group	Patient, No. (%)	Mean or Median Age (±SD or IQR), y	Men, No. (%)	ICU Admission, No. (%)	Transplant Recipients, No. (%)	Immunosuppressant User, No. (%)	ESRD on Hemodialysis, No. (%)	Intravascular Device, No. (%)	Effective Empirical Therapy, No. (%)	Days of Treatment, Median (IQR)	Days of Hospital Stay, Median (IQR)	Mortality, No. (%)
Wiggers et al. [18]	All patients	901 (100)	67 (18)	408 (45)	240 (27)	ND	ND	ND	ND	ND	ND	14 (5–33)	131 (15)[k]
	FUBC	241 (27)	62 (18)	94 (39)	96 (40)	ND	ND	ND	ND	ND	ND	24 (11–46)	35 (15)[k]
	No FUBC	660 (73)	69 (18)	314 (48)	144 (22)	ND	ND	ND	ND	ND	ND	10 (4–29)	96 (15)[k]

Abbreviations: ESRD, end-stage renal disease; FUBCs, follow-up blood cultures; ICU, intensive care unit; IQR, interquartile range; ND, no date.

[a]Patients with ESRD. No data existed on whether all these patients received dialysis.

[b]Central venous catheter.

[c]No details of immunosuppressants were given in the article.

[d]The number (%) of patients with solid organ transplantation was 39 (3) in the whole study population, 34 (3) in patients with FUBCs, and 5 (2) in patients without FUBCs. The number (%) of patients with bone marrow transplantation was 29 (2) in the whole study population, 28 (2) in patients with FUBCs, and 1 (<1) in patients without FUBCs.

[e]Steroid use.

[f]The number (%) of patients who received hemodialysis was 37 (2) in the whole study population, 35 (3) in patients with FUBCs, and 2 (1) in patients without FUBCs. The number (%) of patients who received peritoneal dialysis was 6 (<1) in the whole study population, 6 (<1) in patients with FUBCs, and 0 (0) in patients without FUBCs. These data were extracted from the individual patient data.

[g]There was no mention of specific transplant details (eg, solid organ transplant or bone marrow transplant).

[h]The original article mentioned "days to effective therapy: 0 days."

[i]The number (%) of deaths from attributable causes was 176 (10) in the whole study population, 98 (8) in patients with FUBCs, and 78 (15) in patients without FUBCs.

[j]Only data after propensity score matching were presented.

[k]The number (%) of deaths in the hospital was 193 (21) in the whole study population, 54 (22) in patients with FUBCs, and 139 (21) in patients without FUBCs.

Table 3. Acquisition of FUBC and FUBC Results

Author	Acquisition of FUBCs, No. (%)	Detection of the Same Bacteria in FUBCs, No.	Detection of Total Different Bacteria in FUBCs, No. (%)	Detection of Different True Pathogens in FUBCs, No. (%)	Detection of Possible Contaminants in FUBCs, No. (%)	Negative Results of FUBCs, No. (%)
Amipara et al. [7]	219 (29)	15 (7)	ND	ND	ND	204 (93)
Chan et al. [19]	299 (89)	37 (12)	17 (6)	9 (3)[a]	8 (3)[a]	250 (84)
Elamin et al. [11]	321 (67)	9 (3)	3 (<1)	2 (<1)	1 (<1)	309 (96)
Giannella et al. [9]	278 (18)	107 (38)	28 (10)	17 (6)	11 (4)[b]	143 (51)
Green et al. [16]	127 (80)	9 (7)	9 (7)	ND	ND	109 (86)
Jung et al. [17]	1276 (86)	122 (10)	8 (1)	8 (1)	0 (0)	1146 (90)
Maskarinec et al. [8]	1164 (68)	228 (20)	51 (4)	29 (2)	22 (2)[c]	885 (76)
Mitaka et al. [10]	271 (72)	27 (10)	ND	ND	ND	244 (90)
Wiggers et al. [18]	241 (27)	27 (11)	20 (8)	12 (5)	8 (3)[d]	194 (80)

Abbreviations: FUBCs, follow-up blood cultures; ND, no data.

[a]Three different true pathogens and 2 contaminants were detected simultaneously in patients with the same bacteria in FUBCs.

[b]Coagulase-negative staphylococci, *Corynebacterium* spp., *Propionibacterium* spp.

[c]Coagulase-negative *Staphylococcus*, viridans group *Streptococcus*, and diphtheroids.

[d]Coagulase-negative staphylococci, diphtheroids, or *Bacillus* spp.

between use vs nonuse of FUBCs collected largely within 1 week of disease onset, short-term mortality, and other patient-relevant clinical outcomes. Recently, Thaden et al. also published a systematic review and meta-analysis of the association between FUBCs and mortality in GNB bacteremia [27]. Their study reviewed not only the association between FUBC acquisition and mortality, but also FUBC results and mortality. Our study only evaluated FUBC acquisition; we performed IPD analysis for the 2 original studies. In addition, we evaluated the association between FUBC acquisition and length of hospital stay and treatment.

Our random-effects meta-analysis suggested that FUBCs were associated with a reduced death risk by an average of 43%. FUBC group patients appeared to require a longer treatment duration (median difference, 2–5) and longer hospital stay (median difference, 2–14) than those in the no-FUBC group.

The strengths of this systematic review are 3-fold. First, we followed an explicit, prespecified research question and recommended systematic review methods for observational studies [28], including a comprehensive literature search, dual screen and dual selection of eligible studies, dual extraction of data, dual assessment of bias risk, and random-effects model meta-analysis. Second, we obtained IPD to calculate confounding-corrected results for studies that reported nonadjusted data, which is another recommended method for assessing data from observational studies [26]. Finally, we critically appraised the limitations of the published reports on FUBC use to improve patient-relevant clinical outcomes. Our formal and rigorous assessment of available clinical evidence corroborates and extends previous research [6, 12, 13].

There are multiple reasons why FUBC acquisition may be related to low mortality. First, when FUBCs showed bacteria different from those in the initial blood culture, the antimicrobial agents were changed to appropriate ones, which may have improved the prognosis. However, since the actual proportion of detections of different bacteria was as small as 1%–6% [8, 9, 11, 17–19], the impact this had on mortality was also small. Second, a positive FUBC result may have led to a search for sources that require control or, if there were sources, aggressive control, which may have improved the prognosis [29]. However, sufficient data were not available to explain these rationales. Third, the inability to completely adjust biases was also a major factor. This is discussed in more detail in the Limitations section below.

Secondary outcomes included the length of hospitalization and treatment. Studies have shown a trend toward longer treatment and hospitalization durations in patients with compared with those without FUBCs. There are 2 possible reasons for this. First, treatment and hospitalization may continue, sometimes unnecessarily, while waiting for FUBC results. Second, treatment may have been administered even if the FUBC results showed contamination. However, there were no data to support these hypotheses other than that more patients with FUBCs received source control.

This review has several limitations. All the included studies were observational in nature, and the derived data from clinical practice were deemed to be of critical risk bias. Although some

variables that could cause confounding by indication were adjusted for, bias due to residual confounding and other biases stemming particularly from participant selection, intervention classification, and missing data remained. Judging by the observed clinical heterogeneity, the direction and magnitude of these biases vary across studies and are difficult to predict.

For example, the timing and duration allowed for collecting repeat blood cultures in the study-specified FUBC definitions differed. However, such variations may reflect different testing objectives, at least partially. A very early repeat blood culture (eg, 1–2 days post–first positive blood culture) could have been performed as part of a routine testing strategy, given that no or only limited information on the causative bacterium might have been available. Conversely, a late repeat blood culture (eg, 6–7 days post–first positive blood culture) could have been performed, for example, as a formal assessment for clinically unfavorable responders to ongoing treatment [12]. Additionally, patients with a very poor prognosis in critical condition (eg, those with severe sepsis with a high likelihood of very early death) could have received a routine FUBC conducted just after the first results, but the likelihood would have been too low for recipients of a late FUBC or even participants included in such studies [30]. Thus, these different definitions of the timing of collection may have been associated with selective group assignment, which could have led to selection bias and additional confounding. The effect of these variations on the selection of subsequent treatments for management would highly hinge upon the timing, test objective, and FUBC yield [12]. Furthermore, time-varying confounding may have also been present, where patients who were not scheduled to receive FUBCs received them due to poor clinical course. However, none of the studies addressed this time-dependent confounder. Unfortunately, because of the nature of practice-based data, these theoretically expected biases and variations were not easily addressable, even with statistical adjustments for baseline confounders based on the IPD. Therefore, although consistently positive through the sensitivity analyses, our results are not precisely adjusted confident effect estimates applicable to the bedside, but should be viewed as a guide to design future studies.

Given their uncertain effectiveness, FUBCs of patients with GNB bacteremia remain an unproven test to be assessed in research settings. Conducting sufficiently powered randomized clinical trials comparing an FUBC-based strategy with conventional management is the gold standard for reliably assessing the comparative effectiveness of routine utilization of FUBCs. In this framework, full details of testing strategies, including the objective, timing, and number of FUBCs, should be explicitly formulated in accordance with specific research questions relevant to specific clinical contexts and outcomes. One such example would be upfront testing as an early response assessment and response-oriented treatment modifications for all patients with GNB bacteremia. Another could be late testing exclusively performed for slow or poor responders to empirical treatment, the results of which should help determine salvage treatments. However, given the limited evidence on the diagnostic and prognostic values of FUBCs collected at specific time points [6, 12, 13] and the excessive cost of conducting randomized trials de novo, the prospective registration of all patients with GNB bacteremia and detailed documentation of the aforementioned prespecified clinical information on the application of FUBCs constitute another feasible approach. The use of recently proposed analytical methods coupled with advanced modeling techniques to obtain accurate effect estimates using real-life observational data would also constitute a potential and realistic next step [31, 32].

CONCLUSIONS

Limited data from retrospective studies of heterogeneous and mostly non-neutropenic patient populations showed that FUBCs were associated with lower mortality. Longer hospital stay and treatment duration in hospitalized patients with GNB bacteremia were also observed, although this was not a result of the meta-analysis. However, the risk of bias was critical in all the studies, and no firm data were available to support these mechanisms. Given its uncertain effectiveness, FUBC in patients with GNB bacteremia remains an unproven test to be assessed in future research settings.

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References

1. Goto M, Al-Hasan MN. Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe. Clin Microbiol Infect **2013;** 19:501–9.
2. Diekema DJ, Beekmann SE, Chapin KC, et al. Epidemiology and outcome of nosocomial and community-onset bloodstream infection. J Clin Microbiol **2003;** 41: 3655–60.
3. Kang CI, Kim SH, Park WB, et al. Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. Antimicrob Agents Chemother **2005;** 49:760–6.
4. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. Clin Infect Dis **2011;** 52:e18–55.
5. Pappas PG, Kauffman CA, Andes DR, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis **2016;** 62:e1–50.

6. Cogliati Dezza F, Curtolo A, Volpicelli L, et al. Are follow-up blood cultures useful in the antimicrobial management of gram negative bacteremia? A reappraisal of their role based on current knowledge. Antibiotics (Basel) **2020;** 9:895.
7. Amipara R, Winders HR, Justo JA, et al. Impact of follow up blood cultures on outcomes of patients with community-onset gram-negative bloodstream infection. EClinicalMedicine **2021;** 34:100811.
8. Maskarinec SA, Park LP, Ruffin F, et al. Positive follow-up blood cultures identify high mortality risk among patients with gram-negative bacteraemia. Clin Microbiol Infect **2020;** 26:904–10.
9. Giannella M, Pascale R, Pancaldi L, et al. Follow-up blood cultures are associated with improved outcome of patients with gram-negative bloodstream infections: retrospective observational cohort study. Clin Microbiol Infect **2020;** 26:897–903.
10. Mitaka H, Fujitani S, Kuno T, Perlman DC. Association between follow-up blood cultures for gram-negative bacilli bacteremia and length of hospital stay and duration of antibiotic treatment: a propensity score-matched cohort study. Infect Control Hosp Epidemiol **2022**:1–6. https://doi.org/10.1017/ice.2022.110
11. Elamin A, Khan F, Abunayla A, Jagarlamudi R. 288. Follow-up blood cultures in gram-negative bacteremia: how do they impact outcomes. Open Forum Infect Dis **2020;** 7(Suppl 1):S144.
12. Wiggers JB, Daneman N. The culture of follow-up blood cultures. Clin Microbiol Infect **2020;** 26:811–3.
13. Fabre V, Sharara SL, Salinas AB, et al. Does this patient need blood cultures? A scoping review of indications for blood cultures in adult nonneutropenic inpatients. Clin Infect Dis **2020;** 71:1339–47.
14. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ **2021;** 372:n71.
15. Sterne JA, Hernán MA, Reeves BC, et al. Robins-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ **2016;** 355:i4919.
16. Green A, Liang Y, O'Hara L, et al. Follow-up blood cultures in *Pseudomonas aeruginosa* bacteremia: a potential target for diagnostic stewardship. Antimicrob Steward Healthc Epidemiol **2021;** 1:E23.
17. Jung J, Song KH, Jun KI, et al. Predictive scoring models for persistent gram-negative bacteremia that reduce the need for follow-up blood cultures: a retrospective observational cohort study. BMC Infect Dis **2020;** 20:680.
18. Wiggers JB, Xiong W, Daneman N. Sending repeat cultures: is there a role in the management of bacteremic episodes? SCRIBE study. BMC Infect Dis **2016;** 16: 286.
19. Chan JD, Ta A, Lynch JB, Bryson-Cahn C. Follow-up blood cultures in *E. coli* and *Klebsiella* spp. bacteremia-opportunities for diagnostic and antimicrobial stewardship. Eur J Clin Microbiol Infect Dis **2021;** 40:1107–11.
20. Rothman KJ, Greenland S, Lash TL. Modern Epidemiology. 3th ed. Lippincott Williams & Wilkins; **2008**.
21. Higgins JPT, Thompson SG, Spiegelhalter DJ. A re-evaluation of random-effects meta-analysis. J R Stat Soc A **2009;** 172:137–59.
22. Weber F, Knapp G, Glass Ä, Kundt G, Ickstadt K. Interval estimation of the overall treatment effect in random-effects meta-analyses: recommendations from a simulation study comparing frequentist, Bayesian, and bootstrap methods. Res Synth Methods **2021;** 12:291–315.
23. Riley RD, Higgins JP, Deeks JJ. Interpretation of random effects meta-analyses. BMJ **2011;** 342:d549.
24. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med **2002;** 21:1539–58.
25. Sterne JA, Sutton AJ, Ioannidis JP, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ **2011;** 343:d4002.
26. Faria R, Hernandez Alava M, Manca A, Wailoo A. The use of observational data to inform estimates of treatment effectiveness in technology appraisal: methods for comparative individual patient data, NICE DSU technical support document. NICE **2015**. Available at: https://www.sheffield.ac.uk/nice-dsu/tsds/observational-data
27. Thaden JT, Cantrell S, Dagher M, et al. Association of follow-up blood cultures with mortality in patients with gram-negative bloodstream infections: a systematic review and meta-analysis. JAMA Netw Open **2022;** 5:e2232576.
28. Arroyave WD, Mehta SS, Guha N, et al. Challenges and recommendations on the conduct of systematic reviews of observational epidemiologic studies in environmental and occupational health. J Expo Sci Environ Epidemiol **2021;** 31:21–30.
29. Tungsiripat M. Follow-up blood cultures are often needed after bacteremia. Cleve Clin J Med **2019;** 86:93–4.
30. Yadav K, Lewis RJ. Immortal time bias in observational studies. JAMA **2021;** 325: 686–7.
31. Hernán MA, Robins JM. Using big data to emulate a target trial when a randomized trial is not available. Am J Epidemiol **2016;** 183:758–64.
32. Mansournia MA, Etminan M, Danaei G, Kaufman JS, Collins G. Handling time varying confounding in observational research. BMJ **2017;** 359:j4587.