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Antibiotic use and outcome in patients with negative blood cultures, a new target population for antimicrobial stewardship interventions: A prospective multicentre cohort (NO-BACT)

José Antonio Girón-Ortega [a], Raquel Fernández-Guerrero [a], Montserrat Montes de Oca Arjona [b], Fátima Galán-Sanchez [c], Galadriel Pellejero Sagastizábal [d,e], Elena Morte Romea [d,e,f], Marina de Cueto [f,g,h,i], Margarita Beltrán Garcia [j], Zaira Palacios-Baena [f,g,h,i], Silvia Jiménez Jorge [k], Jesús Rodríguez-Baño [f,g,h,i,*,1], Pilar Retamar-Gentil [f,g,h,i,*,1], NO-BACT Team [2]

[a] *Internal Medicine Department, Hospital Universitario Virgen Macarena, Sevilla, Spain*
[b] *Servicio de Medicina Interna, Enfermedades Infecciosas y Cuidados Paliativos, Hospital Universitario Puerta del Mar, Instituto para la Investigación e Innovación Biomédica de Cádiz (INiBICA), Cádiz, Spain*
[c] *Servicio de Microbiología, Hospital Universitario Puerta del Mar, Instituto para la Investigación e Innovación Biomédica de Cádiz (INiBICA), Cádiz, Spain*
[d] *Serviciode Enfermedades Infecciosas, Hospital Clínico Lozano Blesa, Zaragoza, Spain*
[e] *Institutode Investigación Sanitaria Aragón (IIS Aragón), Zaragoza, Spain*
[f] *CIBER de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain*
[g] *Unidad Clínica de Enfermedades Infecciosas y Microbiología, Hospital Universitario Virgen Macarena, Seville, Spain*
[h] *Departamentos de Medicina y Microbiología, Universidad de Sevilla, Seville, Spain*
[i] *Instituto de Biomedicina de Sevilla (IBiS)/CSIC, Seville, Spain*
[j] *Unidad de Farmacia Hospitalaria, Hospital Universitario Virgen Macarena, Seville, Spain*
[k] *Research and Clinical Trials Unit, Hospital Universitario Virgen del Rocío, Seville, Spain*

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SUMMARY

Objectives: To evaluate the appropriateness of antimicrobial treatment and the risk factors for mortality in patients with negative blood cultures (BC), in order to evaluate whether this population would be a suitable target for antimicrobial stewardship (AMS) interventions.
Methods: A multicentre prospective cohort study of patients with negative BC in three Spanish hospitals between October 2018 and July 2019 was performed. The main endpoints were the appropriateness of antimicrobial treatment (evaluated by two investigators according to local guidelines) and 30-day mortality. Cox-regression was performed to estimate the association between variables and 30-day mortality.
Results: Of 1011 patients in whom BC was obtained, these were negative in 803 (79%) and were included; 30-day mortality was 9% (70 patients); antibiotic treatment was considered inappropriate in 299 (40%) of 747 patients evaluated at day 2, and in 266 (46%) of 573 at day 5–7. The variables independently associated with increased risk of 30-day mortality were higher age (HR 1.05; 95% CI 1.03–1.07), neoplasia (HR 2.73; 95% CI 1.64–4.56), antibiotic treatment in the 48 h prior to BC extraction (HR 2.06; 95% CI 1.23–3.43) and insufficient antibiotic coverage at day 2 after BC obtainment (HR 2.35; 95% CI 1.39–4.00). Urinary, catheter and biliary sources of infection were associated with lower risk (HR 0.40; 95% CI 0.20–0.81).
Conclusions: Antimicrobial treatment is frequently inappropriate among patients with negative BC; insufficient antibiotic coverage at day 2 was associated with mortality. These results suggest that patients with negative BC are a suitable population for AS interventions.
Summary: Antimicrobial treatment in patients with negative blood culture was frequently inappropriate, and inappropriate coverage at day 2 was associated with increased risk of death. These

* Correspondence to: Unidad Clínica de Enfermedades Infecciosas y Microbiología, Hospital Universitario Virgen Macarena, Avda Dr Fedriani 3, 41009 Sevilla, Spain.
E-mail addresses: jesusrb@us.es (J. Rodríguez-Baño), pilaretamar@hotmail.com (P. Retamar-Gentil).
[1] These authors contributed equally as senior authors.
[2] A list of authors belonging to the 'NO-BACT team' is included in the Supplementary material.

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data support the consideration of this population as a potential target for antimicrobial stewardship interventions.

Introduction

Blood cultures (BC) are typically obtained in hospital-admitted patients when an invasive infectious disease is suspected. While bacteremic infections have been extensively studied and specialized consultation in these patients is associated with better outcomes,[1] data from patients with negative BC are scarce, despite being the majority of patients from whom BC are obtained.[2–4] To the best of our knowledge, the factors associated with mortality in patients with negative BC have not been comprehensively studied in prospective studies.

Antimicrobial stewardship (AMS) interventions aim to optimize the use of antibiotics in order to improve clinical outcomes and reduce adverse events while selecting the best cost-effective treatment.[5] Patients with bacteremia are well-recognized targets for AMS interventions[1,6]; however, patients with negative BC have been neglected from the perspective of AMS, despite being easily detectable. We hypothesize that antimicrobial treatment may be frequently inappropriate and could potentially result in worse prognosis in patients with negative BC, and therefore they may represent a target population for AS interventions.

The NO-BACT study aimed to (1) assess the quality of antimicrobial treatment in patients with negative BC and (2) identify the mortality predictors in order to provide background data to inform the potential convenience of AS interventions in these patients.

Methods

Study design and participants

The NO-BACT project includes a prospective cohort study performed in three Spanish University Hospitals (University Hospital Macarena in Seville, University Hospital Puerta del Mar in Cadiz and University Hospital Lozano Blesa in Zaragoza) including adult admitted patients with negative BC between October 2018 to July 2019. All three participating hospitals have active AMS activities including the availability of local guidelines for specific empiric and targeted antimicrobial therapy and unsolicited specialized consultation programs for patients with bacteremia, among others. The study protocol was previously published.[7]

The criteria for obtaining BC were similar in the 3 participating centers, including any admitted patient in whom an invasive infectious disease was suspected according to clinical and laboratory data. The first 60 patients with negative BC every month at each participating site were included; eligible patients were detected by daily consulting the list of patients with negative BC 2 days after their obtainment until the monthly needed number was reached. Exclusion criteria included being discharged from the hospital within the first 48 h following BC extraction, microbial growth in BC after day 2, and missing critical data to evaluate the appropriateness of therapy (i.e., no information about source suspected or clinical severity).

The study was approved by the local research ethics committees of the participating hospitals and the Spanish Agency for Medicines. Due to the observational nature of the study and the use of anonymized data, the need to obtain written informed consent was waived. The STROBE guidelines for reporting observational studies were followed[8] (Supplementary Table S1).

Variables and definitions

The primary outcomes were all-cause 30-day mortality after BC extraction and appropriateness of antimicrobial treatment (see below). Covariables collected included demographics (sex, age, hospital), comorbidities and their severity (using the Charlson abbreviated index), type of acquisition and source of the suspected infection, severity of systemic response, laboratory data (C reactive protein, procalcitonin, creatinine), results from microbiological samples other than blood cultures and antimicrobial treatment. Data on systemic response and laboratory results were collected as available at days 0, 2 and 5–7 after the BC extraction. The data were collected by reviewing the electronic clinical records and included in an electronic case report form.

Appropriateness of antimicrobial treatment (or its absence) was assessed according to the local guidelines of each participating hospital at days 2 and 5–7 after BC obtainment by two local investigators expert in AMS, using a specific evaluation form (Appendix S1). The opinion of a third investigator was requested in case of disagreement. The prescriptions were evaluated as appropriate if coverage, dose, duration and administration route were in accordance with the local guidelines,[9–11] or inappropriate otherwise. Because the guidelines did not provide specific recommendations for patients with negative BCs, the evaluation was done with those provided for the suspected syndrome or source of infection.

The participating hospitals used the same technique for BC obtainment, in accordance with the recommendations of Spanish Society of Infectious Diseases and Clinical Microbiology.[12] Two blood extractions from peripheral veins in two different punctures, with an interval between 5 and 10 and 60 min, depending on the patient's clinical condition were obtained. In each extraction, 20 ml (10 ml into an aerobic blood culture bottle and 10 ml into an anaerobic blood culture bottle) should be obtained. BC were considered as false positives (blood culture contamination) when typical commensal skin colonizing bacteria (such as coagulase-negative staphylococci) were isolated in only one blood draw.[13] BC were considered negative if there was no microbial growth detected after 5 days of incubation. The severity of systemic response was defined in 2001 International Sepsis Definitions Conference.[14] Patients were considered as immunocompromised if any of the following criteria were present: solid or hematological neoplastic disease, use of immunosuppressive treatment including corticosteroids (20 mg of prednisone or equivalent during two or more weeks), and human immunodeficiency virus infection with < 500 T CD_4 cells/μL. Acquisition of infection was defined as nosocomial if symptoms appeared after 48 h of hospital admission or in less than 7 days after hospital discharge.[15] Community-onset infections were considered healthcare-associated if fulfilling Friedman's criteria[16] and community-acquired otherwise.

Statistical analysis

We estimated a 5–10% mortality rate among patients with negative BC; in order to be able to include 4–8 risk factors in a multivariable analysis, we planned to include 800 patients, for which around 1000 patients with BC performed would need to be checked, considering a positivity rate of 20%.

Quantitative variables were expressed as mean and standard deviation or median and interquartile range if not normally

distributed. Qualitative variables were expressed as absolute numbers and percentages. A Kaplan-Meier curve was plotted for 30-day mortality. Crude and multivariable analysis for mortality was performed with patients evaluated at day 2 by Cox regression; all variables with p < 0.10 in the crude analysis were included in the multivariable model, and selected using a stepwise manual backward selection process in which the variables with lower association with mortality was removed at each step; variables with P value <0.10 were retained. Interactions were evaluated. Sensitivity analysis excluding patients in whom antibiotic treatment at day 2 was deemed unnecessary and in those in whom C-reactive protein was measured were also performed. Additionally, factors associated with antibiotic appropriateness were evaluated by logistic regression. Finally, the concordance of paired evaluations for treatment appropriateness was evaluation by the Cohen's kappa statistic. All the analyses were performed using SPSS Statistics 24.0 (IBM, Armonk, NY, USA).

Results

Baseline characteristics

Overall, 1011 patients from whom BC were obtained were evaluated; 803 (79.4%) had negative BC and were included in the study; 283 (35.2%) were included from University Hospital Puerta del Mar, 283 (35.2%) from University Hospital Lozano Blesa and 237 (29.5%) from University Hospital Macarena. The overall positive BC rate was 20.6%; the specific rates per site were 17.1%, 19.1% and 24.5% at Virgen Macarena University Hospital, Lozano Blesa University Hospital and Puerta del Mar University Hospital, respectively; the rates of positivity according to the suspected source of infection are shown in Supplementary Table S2. Their baseline characteristics and the features of the suspected infections are shown in Table 1; overall, 454 patients (56.5%) were male, and the median age was 66 (IQR 54–77); 270 (33.6%) were considered immunocompromised. The suspected infection was community-acquired in 320 patients (39.9%), and community-onset but healthcare-associated in 208 (25.9%). The most frequent source of the suspected infection was pneumonia (260 patients, 32.3%); 104 (12.9% patients presented with severe sepsis or septic shock. Overall, 293 patients (36.5%) were receiving antimicrobial treatment in the 48 h prior to blood cultures obtainment.

Antibiotic treatments and quality of prescriptions

Antimicrobial treatment (or its absence) was evaluated in 747 patients at day 2 (93.0%) and in 573 at day 5–7 (71.3%); the reason for not being evaluated at day 2 was death in 6 (0.8%) and previous discharge in 50 (6.2%); and at day 5–7 (in addition to these), death in 16 (2.0%), previous discharge in 141 (17.5%) and missing critical data in 17 (2.1%).

Overall, the number of patients receiving antibiotics at the different time points was: 666 patients of the 803 patients (82.9%) at day 0; 654 of 747 on day 2 (87.5%); and 544 of 573 (94.9%) on day 5–7 (Table 2). Combination therapy was administered to 177 patients at day 0 (22.0% of patients), 183 (24.4%) at day 2 and 137 (23.9%) at day 5–7. In total, 130 antibiotics (19.5% of prescriptions at day 0) which were started in the 48 h prior to BC obtainment were continued after the BC extraction. The most frequent antibiotics used in monotherapy at day 0 were: ceftriaxone in 134 patients (16.7% of patients), piperacillin-tazobactam in 98 patients (12.2%) and amoxicillin-clavulanic acid in 92 (11.5%); at day 5–7, these drugs were being used in 72 (12.6%), 84 (14.6%) and 72 (12.6%), respectively (Table 2).

Overall, antimicrobial prescriptions (or their absence) were considered inappropriate in 299 patients at day 2 (40.0% of evaluated patients), and in 266 (46.4%) at day 5–7. A Cohen's Kappa of 0.95 was obtained for prescriptions assessment by paired evaluators, which indicates a very good level of concordance.

The reasons for inappropriateness (Fig. 1) were, at day 2: antibiotic(s) were not necessary in 136 patients (18.2% of those evaluated), excess of coverage in 111 (14.8%), insufficient coverage in 130

Table 1
Baseline characteristics of 803 patients with negative blood cultures and their suspected infection.

Variable	No. of patients (%) or median (IQR)
Demographic characteristics	
Median age in years (IQR)	66 (54–77)
Male sex	454 (56.5)
Comorbidities	
Abbreviated Charlson index, median (IQR)	2 (0–2)
Diabetes mellitus	213 (26.5)
Heart failure/ischemic heart disease	168 (20.9)
Dementia	90 (11.2)
Pulmonary chronic disease	118 (14.7)
Cerebrovascular disease	80 (9.9)
Peripheral arterial disease	48 (5.9)
Immunocompromised	270 (33.6)
Solid cancer	144 (17.9)
Hematological cancer	61 (7.5)
Chronic kidney disease with substitutive therapy	94 (11.7)
Indwelling devices	
Peripheral venous catheter	241 (30.0)
Central venous catheter	88 (11.0)
Peripherally inserted central venous catheter	24 (2.9)
Urinary catheter	130 (16.1)
Vascular/valvular prosthesis	46 (5.7)
Invasive mechanical ventilation	46 (5.7)
Prosthetic joint	24 (2.9)
Double J stent	7 (0.9)
Nephrostomy	11 (1.3)
Biliary prosthesis	9 (1.1)
Nasogastric tube	32 (3.9)
Pacemaker/automatic defibrillator implant	26 (3.2)
Percutaneous endoscopic gastrostomy	7 (0.2)
Clinical data of suspected infection (reason for blood cultures extraction)	
Type of acquisition	
Community-acquired	320 (39.9)
Community-onset, health-care related	208 (25.9)
Nosocomial	275 (34.2)
Suspected site of infection	
Pneumonia	260 (32.3)
Urinary tract	146 (18.2)
Intraabdominal (excluding biliary tract)	71 (8.8)
Biliary tract	47 (5.9)
Vascular catheter	28 (3.5)
Skin and skin structures	39 (4.9)
Osteoarticular	5 (0.6)
Multiple sites	58 (7.2)
Unknown	102 (12.7)
Others	47 (5.8)
Systemic response severity at blood cultures obtainment	
Severe sepsis	77 (9.6)
Septic shock	27 (3.3)
Laboratory data at blood cultures obtainment	
Creatinine, median (IQR) mg/dL (n = 701, 87%)	0.9 (0.7–1.4)
C reactive protein, median (IQR) mg/L (n = 619, 77%)	65.9 (15.1–177.1)
Procalcitonin, median (IQR) ng/ml (n = 181, 23%)	0.42 (0.2–2.0)
Microbiological findings	
Additional microbiological samples obtained, other than blood cultures	513 (63.8)
Urine	286 (55.8)
Sputum or bronchoalveolar lavage	49 (9.6)
Stool	18 (3.5)
Positive microbiological samples other than blood cultures	201 (39.1)
Antimicrobial treatment in the 48 h prior to blood culture extraction	293 (36.5)

IQR: interquartile range.

Table 2
Antibiotic treatments at days 0 (blood cultures obtainment), 2 and 5–7.

Antibiotic administered	Day 0 (n = 803)	Day 2 (n = 747)	Day 5–7 (n = 573)
Any antibiotic	666 (82.9)	654 (87.5)	544 (94.9)
Monotherapy	489 (60.9)	471 (63.1)	407 (71.0%)
Ceftriaxone	134 (16.7)	127 (17.0)	72 (12.6%)
Piperacillin/tazobactam	98 (12.2)	102 (13.7)	84 (14.6%)
Amoxicillin/clavulanic acid	92 (11.5)	77 (10.3)	72 (12.6%)
Meropenem	50 (6.2)	52 (6.9)	47 (8.2%)
Levofloxacin	39 (4.9)	32 (4.2)	30 (5.2%)
Others	76 (9.5)	81 (10.8)	102(17.8%)
Combination therapy	177 (22.0)	183 (24.4)	137 (23.9)
Most common combinations			
Ceftriaxone + levofloxacin	20 (2.5)	18 (2.4)	9 (1.5)
Ceftriaxone + azithromycin	12 (1.5)	12 (1.6)	5 (0.8)
Ceftriaxone + metronidazole	10 (1.2)	11 (1.5)	8 (1.3)
Meropenem + linezolid	10 (1.2)	13 (1.7)	12 (2.1)

(17.4%), route in 25 (3.3%) and dose in 41 (5.4%). At day 5–7, the reasons were: antibiotic(s) were not necessary in 147 (25.6%), excess of coverage in 104 (18.1%), insufficient coverage in 67 (11.6%), route in 88 (15.3%), dose in 20 (3.4%), and duration in 15 (2.6%). The appropriateness of treatment at day five according to evaluation at day 2 is specified in Supplementary material, Fig. S1.

Additionally, the association of different variables with antimicrobial inappropriateness was evaluated (Supplementary Table S3). In the adjusted analysis, factors related to antimicrobial inappropriateness at day 2 were antimicrobial treatment in the 48 h prior to blood culture extraction (OR 1.45; CI95% 1.06–1.96) and pneumonia source (OR 1.43; CI95% 1.04–1.96). At day 5–7 the variables were pneumonia (OR 2.17; CI95% 1.47 – 3.13) and urinary tract infection (OR 2.51; CI95% 1.56 – 3.85). Due to its relevance, the analysis was also performed for insufficient antibiotic coverage at day 2; the variables independently associated with it were nosocomial or healthcare-related acquisition (adjusted OR 2.69; 95% CI 1.70–4.24) and severe sepsis or shock on day 0 (adjusted OR 2.56; CI95% 1.57 – 4.17). Regarding the evaluation of specific drugs, there was a higher proportion of inappropriate treatments with levofloxacin at day 2, and of ceftriaxone, at days 5–7; all data about the appropriateness of antibiotic treatment on day 2 and days 5–7 for the different antimicrobial agents are shown in Supplementary Material (Table S4).

A specific analysis of the reasons for inappropriate treatment in the two main sites of infection (pneumonia and urinary tract infection [UTI]) is shown in Supplementary Data (Table S5). Overall, on day 2 the proportion of unnecessary treatments at day 2 in patients with pneumonia and UTI was lower than in the whole cohort. Patients with pneumonia had a higher frequency of excess of coverage, while patients with UTI had a higher proportion of insufficient coverage. On day 5, both infections presented a lower proportion of unnecessary treatments and a higher proportion of excess of coverage and inappropriate route.

Outcome analysis

Overall, 70 patients died (8.7% of all patients with negative BC); the Kaplan-Meier survival curve is shown in Supplementary Data, Fig. S2. The median hospital length of stay until discharge or death was 8 days (IQR 5-15). The variables with a significant association with mortality in the unadjusted analysis (Table 3) were higher age, abbreviated Charlson index ≥1, diabetes mellitus, heart failure/ischemic heart disease, dementia, cerebrovascular disease, neoplasia, immunocompromised patients, severe sepsis/septic shock, healthcare-related or nosocomial acquisition, receipt of antibiotics during the 48 h prior to BC extraction and insufficient antibiotic coverage at day 2.

For multivariable analysis, the sources of infections were grouped into low-risk sites (including urinary, catheter or biliary tract infections) and all others. The factors independently associated with increased risk of 30-day mortality in Cox regression analysis (performed with 747 patients, 93.0%) were age, neoplasia, antibiotic treatment received in the 48 h prior to BC extraction and insufficient antibiotic coverage on day 2 after BC obtainment, while low-risk site of infection was associated with lower risk of death (Table 4). No significant interactions were detected.

Sensitivity analyses were performed excluding patients in whom antibiotic treatment at day 2 was deemed unnecessary (n = 611, 76.1%) and in patients with C-reactive protein levels measured at day 0 (n = 565, 70.3%); the Cox regression models included the same variables as in the whole population analysis (Supplementary Material, Tables S6 and S7); a higher C-reactive protein level was also associated with a higher hazard of mortality.

Discussion

In this study, we found that antimicrobial treatment in patients with negative BC was almost universal and frequently inappropriate and that mortality in these patients was independently associated with insufficient antibiotic coverage at day 2 after BC obtainment. These findings strongly suggest that this population would be worth being explored as a potentially convenient target for AMS interventions. The percentage of negative blood cultures in our series is similar to what has been published by other authors.[3]

When trying to put the outcome analysis in context, we could not find published studies in which the risk factors for mortality were prospectively assessed in patients with negative blood cultures. Therefore, we used studies analyzing patients with sepsis because a proportion of those patients had negative BC. Similar to our results, older age has been associated with mortality in patients with sepsis.[17,18] With regards to comorbidities, some of them have also been previously found to be strongly associated with mortality.[19] We used the abbreviated Charlson index, which includes 8 variables,[20] and found that punctuation ≥1 in this easy-to-calculate index was associated with increased mortality in unadjusted; however, for the multivariable analysis we decided to include the specific comorbidities rather than the index, trying to identify the specific group of patients who would benefit more of an eventual AMS intervention, and we found that patients with cancer were at increased risk of death. This variable has been previously found to be associated with worse outcomes in patients with severe sepsis/septic shock.[21]

Mortality in patients with sepsis varies depending on the definition utilized. We decided to use the “classical definition” (SEPSIS-1) to facilitate the comparison with previous studies.[14] Fleischmann et al. found a mortality rate of 26% (95% CI, 20–33%) in patients with severe sepsis or septic shock.[22] The mortality rate in our study in patients with severe sepsis or shock (21%) was in the low range of Fleischman´s estimations, probably reflecting the fact that BC were negative since bacteremia had been frequently associated with higher mortality.[3,23–25] Also, Kaukonen et al., in a study including more than 100,000 patients, found that mortality increased lineally with each criterion of the systemic inflammatory response syndrome.[26] In our analysis, we had a low proportion of patients with severe sepsis or septic shock probably because of the exclusion of bacteremic patients, which limited the statistical power of our analysis for this variable; nevertheless, these patients had a clear trend toward increased risk of death. Anyway, we think that BC-negative patients with data suggestive of severe sepsis or shock should prompt a rapid evaluation.

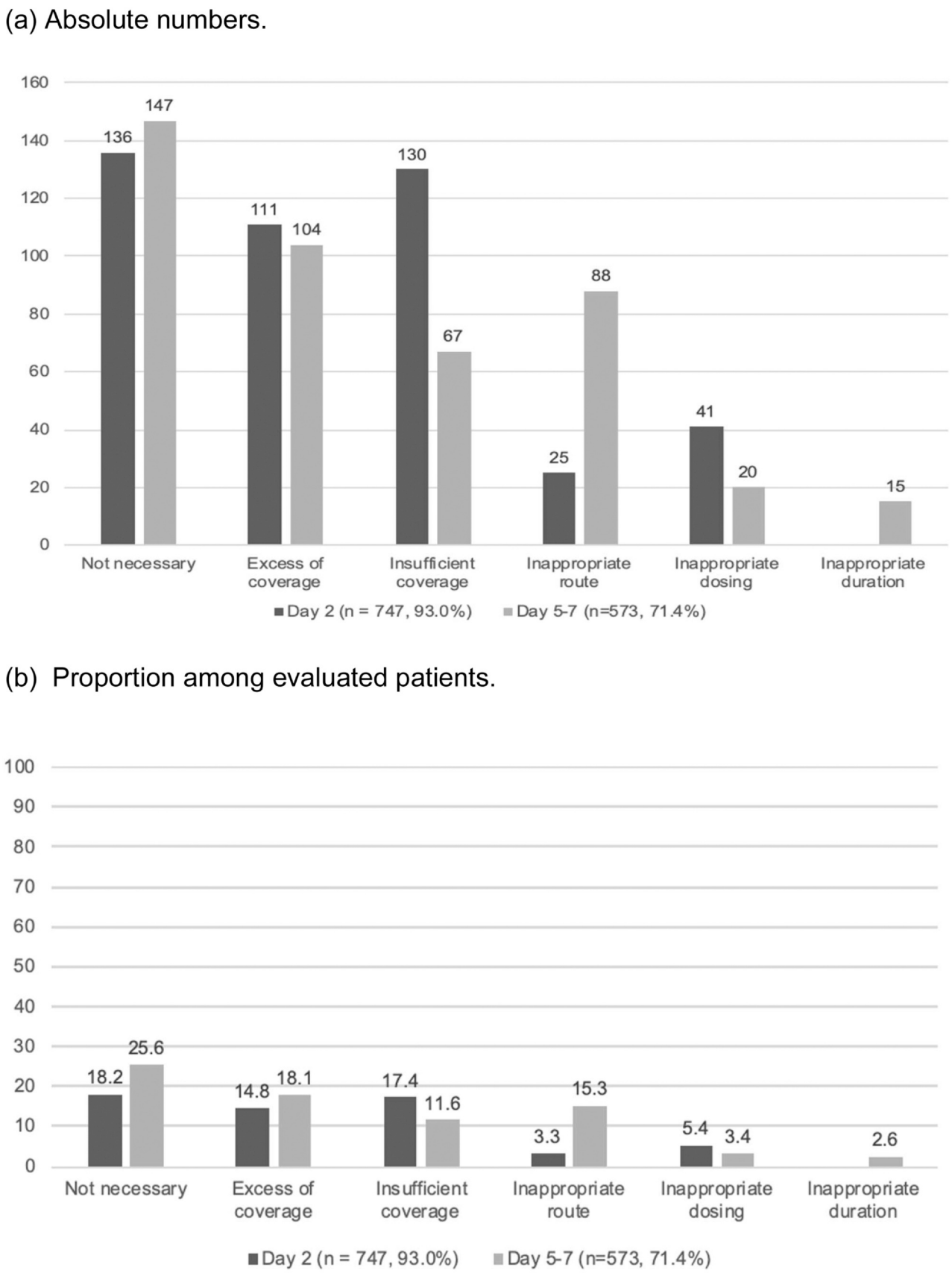

Fig. 1. Reasons for inappropriate therapy among patients with negative blood cultures at days 2 and 5–7. Overall, treatment was inappropriate in 299 (40.0%) patients at day 2 and 266 (46.4%) patients at day 5–7. (a) Absolute numbers. (b) Proportion among evaluated patients.

Table 3
Crude analysis of the association of variables and 30-day mortality in patients with negative blood culture, performed by Cox-regression.

Variables	Alive N = 685	Dead N = 62	Crude HR (95% CI)	p value
Demographic characteristics and comorbidities				
Male sex	384 (56.1)	42 (67.7)	1.60 (0.94–2.73)	0.08
Median age in years	65.0	75.5	1.04 (1.02–1.06)	< 0.001
Abbreviated Charlson index ≥1	476 (69.5)	58 (93.5)	6.04 (2.19–16.63)	< 0.001
Diabetes mellitus	172 (25.1)	27 (44.5)	2.19 (1.33–3.62)	0.002
Heart failure/ ischemic heart disease	142 (20.7)	19 (30.6)	1.66 (0.97–2.86)	0.06
Dementia	72 (10.5)	12 (19.4)	1.96 (1.05–3.68)	0.04
Cerebrovascular disease	64 (9.3)	13 (21.0)	2.38 (1.29–4.39)	0.01
Neoplasia	162 (23.6)	28 (45.2)	2.51 (1.52–4.14)	< 0.001
Pulmonary chronic disease	104 (15.2)	11 (17.7)	1.21 (0.63–2.32)	0.57
Chronic kidney disease with substitutive therapy	84 (12.3)	7 (11.3)	0.91 (0.41–1.99)	0.81
Immunocompromised patient	229 (33.4)	29 (46.8)	1.69 (1.02–2.78)	0.04
Severity and type of acquisition				
Severe sepsis/septic shock	84 (12.3)	15 (24.2)	2.23 (1.24–3.98)	0.01
Healthcare-related or nosocomial acquisition	408 (59.6)	51 (82.3)	3.15 (1.61–6.15)	< 0.001
Site of infection				
Biliary tract	46 (6.7)	1 (1.6)	0.23 (0.03–1.70)	0.15
Intraabdominal (except biliary infection)	64 (9.3)	4 (6.5)	0.67 (0.25–1.89)	0.47
Catheter	27 (3.9)	1 (1.6)	0.41 (0.06–2.94)	0.37
Unknown	89 (13.0)	7 (11.3)	0.85 (0.39–1.87)	0.69
Pneumonia	218 (31.8)	24 (38.7)	1.33 (0.79–2.21)	0.27
Skin structures or osteoarticular	33 (4.8)	4 (6.5)	1.33 (0.48–3.67)	0.58
Urinary tract	127 (18.5)	7 (11.3)	0.56 (0.25–1.24)	0.16
Microbiological samples different than BC				
Microbiological samples taken	475 (69.3)	36 (58.1)	0.63 (0.39–1.04)	0.07
Pathogen isolated in sample different from BC	180 (26.3)	17 (27.4)	1.05 (0.59–1.83)	0.88
Treatment				
Antibiotics during 48 h prior to BC extraction	243 (35.5)	33 (53.2)	1.99 (1.21–3.28)	0.01
Antibiotics administered after BC extraction	569 (83.1)	53 (85.5)	1.19 (0.59–2.41)	0.63
Appropriate treatment at day 2	416 (60.7)	32 (51.6)	0.70 (0.43–1.15)	0.16
Insufficient antibiotic coverage at day 2	108 (15.8)	22 (35.5)	2.76 (1.64–4.64)	< 0.001

BC: blood cultures.

Table 4
Multivariate analysis of the association of variables and 30-day mortality in patients with negative blood culture, performed by Cox-regression.

Variable	Adjusted HR (95% CI)	p value
Median age, per year	1.05 (1.03–1.07)	< 0.001
Neoplasia	2.73 (1.64–4.56)	< 0.001
Severe sepsis/septic shock	1.70 (0.93–3.06)	0.08
Low-risk site of infection[1]	0.40 (0.20–0.81)	0.01
Antibiotics during 48 h prior to BC extraction	2.06 (1.23–3.43)	0.01
Insufficient antibiotic coverage at day 2	2.35 (1.39–4.00)	0.002

BC: blood cultures
[*] Includes urinary tract, biliary tract and catheter.

Regarding the site of infection, urinary tract infections and pneumonia are typically associated with better and worse prognosis, respectively, among patients with sepsis.[27] In our study, in order to facilitate the interpretation, we combined the three sites of infection with lower mortality (urinary, catheter, biliary infections), and these low-risk sites were independently associated with lower mortality. This has been traditionally explained by a better concentration of antibiotics at the site of infection and a better possibility of source control. Again, data suggesting sources with higher mortality would be considered a priority for evaluation.

Interestingly, antibiotic use in the 48 h before the blood culture extraction was associated with higher mortality in multivariable analysis. There might be two explanations for that: first, some of these patients might actually be infected by treatment-resistant bacteria, as continuing with signs of infection after starting treatment; and second, the BC might be falsely negative in them because of the use of antibiotics,[28] which would put them at similar risk of death as bacteremic patients. Mellhammar et al. showed similar results to ours about previous antibiotic therapy in non-bacteremic septic patients admitted to intensive care.[29] It is also remarkable that insufficient antibiotic coverage at day 2 after BC obtainment, which occurred in 17.4% of our patients, was associated with two-fold increase in the hazard of mortality in our cohort. Inadequate empirical treatment has been associated with worse prognosis in bacteremic patients[30,31] and in specific syndromes, mainly in pneumonia.[32] We found only one previous study evaluating the impact of inappropriate treatment in patients with negative BC; in a retrospective cohort study by Kethireddy et al., the adequacy of treatment was associated with better prognosis both in patients with positive or negative cultures results.[33] In our study, the variables independently associated with insufficient coverage were presentation with severe sepsis or septic shock and nosocomial or healthcare-related infections, suggesting that patients with these conditions might be prioritized for interventions. These results, if confirmed in other studies, provide an important argument to propose the active evaluation of patients with negative BC at day 2, particularly if other risk factors for mortality are also present.

The scarcity of data in patients with negative BC may be attributed to intrinsic difficulties in evaluating the quality of antibiotic prescription in them, as there is no microorganism to confront the appropriateness of the drugs used unless other samples provide the etiology of the infection. To solve this, we used the local guidelines and considered the clinical syndrome (site of the suspected infection), the severity of inflammatory response at presentation and the result of other microbiological samples when available.

Our study has some limitations. We may have not included some relevant variables as data for patients with negative BC are scarce, and therefore we needed to select the variables to be collected according to studies performed in patients with sepsis or bacteremia. The evaluation of the appropriateness of treatment might be

affected by subjectivity, although we tried to minimize an evaluator bias by using the local guidelines and the opinion of two evaluators. There was a high level of agreement among the evaluators, as indicated by a Cohen´s Kappa index of 0.95. The sample size might have been insufficient to detect a significant association for some variables related to prognosis. Although we excluded patients with critical missing data; the number of excluded patients for this reason was very low, probably because of the prospective nature of the study. Finally, the study was performed in 3 tertiary centers in Spain, and the results might not be extrapolated to other hospitals or geographical regions or using different criteria to evaluate antimicrobial prescriptions.

In conclusion, we found that antimicrobial treatment in patients with negative BC was frequently inappropriate, and found that inappropriate coverage at day 2 was associated with increased risk of death. These data support the consideration of this population as a potential target for AMS interventions. In fact, these results prompted us to initiate a second phase of the NO-BACT project, which is a cluster randomized trial to test an AMS intervention in this patients population.[7]

Transparency declaration

All authors report no conflicts of interest relevant to this article.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2023.11.013.

References

1. Jiménez-Aguilar P, Romero-Palacios A, De-la-Calle IJ, Martínez-Rubio MC, Girón-González JA, Rodríguez-Baño J. *Unsolicited consultation by infectious diseases specialist improves outcomes in patients with bloodstream infection: A prospective cohort study. J Infect* 2018;**77**(6):503–8. Available from: ⟨https://pubmed-ncbi-nlm-nih-gov.bvsspa.idm.oclc.org/30171877/⟩.
2. Gonçalves-Pereira J, Povoa PR, Lobo C, Carneiro AH. *Bloodstream infections as a marker of community-acquired sepsis severity. Results from the Portuguese community-acquired sepsis study (SACiUCI study). Clin Microbiol Infect* 2013;**19**(3):242–8.
3. Panday RSN, Lammers EMJ, Alam N, Nanayakkara PWB. *An overview of positive cultures and clinical outcomes in septic patients: a sub-analysis of the Prehospital Antibiotics Against Sepsis (PHANTASi) trial. Crit Care* 2019;**23**(1):1–9. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/31113475/⟩.
4. Marwick CA, Guthrie B, Pringle JEC, McLeod SR, Evans JMM, Davey PG. *Identifying which septic patients have increased mortality risk using severity scores: a cohort study. BMC Anesth* 2014;**14**:1–9. Available from: ⟨https://pubmed-ncbi-nlm-nih-gov.bvsspa.idm.oclc.org/24383430/⟩.
5. Rodríguez-Baño J, Paño-Pardo JR, Alvarez-Rocha L, Asensio Á, Calbo E, Cercenado E, et al. *Programas de optimización de uso de antimicrobianos (PROA) en hospitales españoles: documento de consenso GEIH-SEIMC, SEFH y SEMPSPH. Enferm Infecc Microbiol Clin* 2012;**30**(1):e1–23. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/22178010/⟩.
6. Vogel M, Schmitz RPH, Hagel S, Pletz MW, Gagelmann N, Scherag A, et al. *Infectious disease consultation for Staphylococcus aureus bacteremia - A systematic review and meta-analysis. J Infect* 2016;**72**(1):19–28. Available from: ⟨https://pubmed-ncbi-nlm-nih-gov.bvsspa.idm.oclc.org/26453841/⟩.
7. Jiménez-Jorge S, Palacios-Baena ZR, Rosso-Fernández CM, Girón-Ortega JA, Rodriguez-Baño J, Retamar P. *Opportunities for antibiotic optimisation and outcome improvement in patients with negative blood cultures: study protocol for a cluster-randomised crossover trial, the NO-BACT study. BMJ Open* 2019;**9**(12):1–8. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/31857298/⟩.
8. Cuschieri S. *The STROBE guidelines. Saudi J Anaesth* 2019;**13**(Suppl 1):S31–4. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/30930717/⟩.
9. Guia de Antibioterapia Hospital Universitario Virgen Macarena. [cited 2023 Feb 3]. Available from: ⟨https://www.hospitalmacarena.es/activos/antibioterapia/⟩.
10. Guía antibioterapia Hospital Universitario Lozano Blesa. [cited 2023 Feb 3]. Available from: ⟨https://guiaantibioticosproahcuz.com/?hcuz⟩.
11. Guía antibioterapia Hospital Universitario Puerta del Mar. [cited 2023 Feb 3]. Available from: ⟨http://guia2019.implemento.es/⟩.
12. Guna Serrano MR, Larrosa Escartín N, Marín Arriaza M, Rodríguez Díaz JC. *Diagnóstico microbiológico de la bacteriemia y la fungemia: hemocultivos y métodos moleculares. Enferm Infecc Microbiol Clin* 2019;**37**(5):335–40.
13. Dawson S. *Blood culture contaminants. J Hosp Infect* 2014;**87**(1):1–10. Available from: ⟨https://pubmed-ncbi-nlm-nih-gov.bvsspa.idm.oclc.org/24768211/⟩.
14. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. SCCM/ESICM/ACCP/ATS/SIS. *2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med* 2003;**31**(4):1250–6. [cited 2020 Sep 26]. Available from:. ⟨https://ws003.sspa.juntadeandalucia.es:2447/12682500/⟩.
15. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. *CDC definitions for nosocomial infections, 1988. AJIC Am J Infect Control* 1988;**16**(3):128–40. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/2841893/⟩.
16. Friedman ND, Kaye KS, Stout JE, McGarry SA, Trivette SL, Briggs JP, et al. *Health care-associated bloodstream infections in adults: A reason to change the accepted definition of community-acquired infections. Ann Intern Med* 2002;**137**(10):791–7. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/12435215/⟩.
17. Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, et al. *Sepsis in European intensive care units: Results of the SOAP study. Crit Care Med* 2006;**34**(2):344–53. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/16424713/⟩.
18. Fleischmann C, Thomas-Rueddel DO, Hartmann M, Hartog CS, Welte T, Heublein S, et al. *Hospital incidence and mortality rates of sepsis. Dtsch Arztebl Int* 2016;**113**(10):159–66. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/27010950/⟩.
19. Ou SM, Chu H, Chao PW, Lee YJ, Kuo SC, Chen TJ, et al. *Long-term mortality and major adverse cardiovascular events in sepsis survivors a nationwide population-based study. Am J Respir Crit Care Med* 2016;**194**(2):209–17. Available from: ⟨www.atsjournals.org⟩.
20. Berkman LF, Leo-Summers L, Horwitz RI. *Emotional support and survival after myocardial infarction: A prospective, population-based study of the elderly. Ann Intern Med* 1992;**117**(12):1003–9. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/1443968/⟩.
21. Tolsma V, Schwebel C, Azoulay E, Darmon M, Souweine B, Vesin A, et al. *Sepsis severe or septic shock: Outcome according to immune status and immunodeficiency profile. Chest* 2014;**146**(5):1205–13. Available from: ⟨https://ws003.sspa.juntadeandalucia.es:2447/25033349/⟩.
22. Fleischmann C, Scherag A, Adhikari NKJ, Hartog CS, Tsaganos T, Schlattmann P, et al. *Assessment of global incidence and mortality of hospital-treated sepsis current estimates and limitations. Am J Respir Crit Care Med* 2016;**193**(3):259–72. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/26414292/⟩.
23. Søgaard M, Nørgaard M, Pedersen L, Sørensen HT, Schønheyder HC. *Blood culture status and mortality among patients with suspected community-acquired bacteremia: A population-based cohort study. BMC Infect Dis* 2011;**11**(1):139. Available from: ⟨http://www.biomedcentral.com/1471-2334/11/139⟩.
24. Lindvig KP, Nielsen SL, Henriksen DP, Jensen TG, Kolmos HJ, Pedersen C, et al. *Mortality and prognostic factors of patients who have blood cultures performed in the emergency department: A cohort study. Eur J Emerg Med* 2016;**23**(3):166–72. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/25710084/⟩.
25. Brooks D, Smith A, Young D, Fulton R, Booth MG. *Mortality in intensive care: The impact of bacteremia and the utility of systemic inflammatory response syndrome. Am J Infect Control* 2016;**44**(11):1291–5. https://doi.org/10.1016/j.ajic.2016.04.214
26. Kaukonen KM, Bailey M, Pilcher D, Cooper DJ, Bellomo R. *Systemic inflammatory response syndrome criteria in defining severe sepsis. N Engl J Med* 2015;**372**(17):1629–38. Available from: ⟨https://ws003.sspa.juntadeandalucia.es:2447/25776936/⟩.
27. Motzkus CA, Luckmann R. *Does infection site matter? A systematic review of infection site mortality in sepsis. J Intensive Care Med* 2017;**32**(8):473–9. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/26880006/⟩.
28. Cheng MP, Stenstrom R, Paquette K, Stabler SN, Akhter M, Davidson AC, et al. *Blood culture results before and after antimicrobial administration in patients with severe manifestations of sepsis: A diagnostic study. Ann Intern Med* 2019;**171**(8):547–54. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/31525774/⟩.
29. Mellhammar L, Kahn F, Whitlow C, Kander T, Christensson B, Linder A. *Bacteremic sepsis leads to higher mortality when adjusting for confounders with propensity score matching. Sci Rep* 2021;**11**(1):1–12. Available from: ⟨https://pubmed-ncbi-nlm-nih-gov.bvsspa.idm.oclc.org/33772090/⟩.

30. Retamar P, Portillo MM, López-Prieto MD, Rodríguez-López F, De Cueto M, García MV, et al. *Impact of inadequate empirical therapy on the mortality of patients with bloodstream infections: a propensity score-based analysis. Antimicrob Agents Chemother* 2012;**56**(1):472–8. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/22005999/⟩.
31. Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. *The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest* 2000;**118**(1):146–55. Available from: ⟨https://pubmed-ncbi-nlm-nih-gov.bvsspa.idm.oclc.org/10893372/⟩.
32. McCabe C, Kirchner C, Zhang H, Daley J, Fisman DN. *Guideline-concordant therapy and reduced mortality and length of stay in adults with community-acquired pneumonia: playing by the rules. Arch Intern Med* 2009;**169**(16):1525–31. Available from: ⟨https://pubmed-ncbi-nlm-nih-gov.bvsspa.idm.oclc.org/19752411/⟩.
33. Kethireddy S, Bilgili B, Sees A, Kirchner HL, Ofoma UR, Light RB, et al. *Culture-negative septic shock compared with culture-positive septic shock: A retrospective cohort study. Crit Care Med* 2018;**46**(4):506–12. Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/29293143/⟩.