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Diagnosis of bacteraemia and growth times

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SUMMARY

Objective: The objective of this study was to predict the diagnosis of bacteraemia as a function of the time at which the automated BacT/Alert system continuously detects microorganism growth.
Methods: A retrospective study of a database of 1334 patients with a positive blood culture between January 2011 and June 2013 was conducted. Together with the final blood culture results and the patient's history, growth was then analysed to assess whether it represented true bacteraemia or bacterial contamination. The earliest detection times of bacterial growth in each batch of blood cultures were analysed in a blinded fashion after classification.
Results: In total, 590 batches of blood cultures corresponded to true bacteraemia and 744 to bacterial contamination. In the bacteraemia group, the median growth time was 12.72 h (interquartile range (IQR) 10.08–17.58 h). In the contaminated blood culture group, the median growth time was 20.6 h (IQR 17.04–32.16 h) ($p < 0.001$). Analysis of the receiver operating characteristics (ROC) curve (area under the curve 0.80, 95% confidence interval 0.771–0.826) showed that 90% of the contaminants grew after 14.7 h (sensitivity 90.5%, specificity 63.4%, positive predictive value (PPV) 65.9%, negative predictive value (NPV) 90.7%). Forty-five percent of the bacteraemia organisms grew in under 12 h (sensitivity 45.3%, specificity 95%, PPV 87.8%, NPV 68.7%). Microorganisms such as *Candida sp* and *Bacteroides sp* presented median growth times significantly longer than those of the other microorganisms. The administration of antibiotics in the week prior to bacteraemia was found to delay the growth time of microorganisms ($p < 0.001$).
Conclusions: Knowing the time to detection of microorganism growth can help to distinguish between true bacteraemia and bacterial contamination, thus allowing more timely clinical decisions to be made, before definitive microorganism identification.

1. Introduction

The diagnosis of bacteraemia is one of the most critical functions of clinical microbiology laboratories. In general, conventional blood culture methods (manual systems such as biphasic blood culture, lysis filtration–centrifugation, manometer methods, and automatic systems, both radiometric and non-radiometric) provide results within 2 to 5 days, and incubation periods of over 5 days are not usually required with modern continuous automated monitoring methods.[1,2] The volume of blood extracted for each blood culture is the most important variable when harvesting bacteria and fungi from patients with bacteraemia.[1–3]

Contamination of blood culture is common, is very costly for the health system, and often confuses clinicians. To minimize the risk of blood culture contamination with the normal skin flora, meticulous attention should be paid when preparing the skin for venipuncture. In general, it is desirable to maintain the rate of contaminated blood culture at less than 3%. Rates of contamination any higher than this should be investigated and corrected with educational efforts.[4]

Clinicians should be notified every time there is a positive blood culture because the microorganism can often represent an infection that may lead to death. Furthermore, knowledge of the start times for adequate antibiotic therapy is critical for the

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prognosis of patients with sepsis and bacteraemia. The association between mortality and the origin of bacteraemia appears to depend on the use and timing of treatment with adequate empirical antibiotics.[5–7]

The objective of this study was to predict the diagnosis of bacteraemia in the face of contaminants as a function of the time at which the automated BacT/Alert system continuously detects microorganism growth, and to identify whether this time to detection can help the clinician to distinguish true-positive growth from contaminated growth. This study also analysed whether taking antibiotics during the 7 days before bacteraemia influences culture growth times.

2. Methods

The study was conducted in a referral hospital serving the population of the southern part of the Community of Madrid. According to data from the National Institute of Statistics dating from January 1, 2012, this facility covers a health area of 224 549 inhabitants, of whom 171 164 are aged >14 years. The hospital is a 350-bed secondary hospital with general surgery, orthopaedics, urology, gynaecology and obstetrics, internal medicine, intensive care, cardiology, digestive system, pneumology, nephrology, oncology, haematology, and paediatric units.

The growth time in hours from the first vial of each blood culture batch in which microorganism growth was produced in a suspected case of bacteraemia was recorded retrospectively for cultures performed during the period January 2011 to June 2013. All subjects were non-paediatric patients aged >14 years. A retrospective study of this database was then performed.

Together with the final blood culture results and the patient's history (146 different clinical, analytical, and epidemiological variables were recovered), the growth and treatment received were analysed in a blinded fashion by four different clinicians to assess whether the growth represented true bacteraemia or bacterial contamination. The data were analysed using SPSS statistical software (version 15.0). The distribution of these times was studied to decide whether to work with the mean and standard deviation or with the median and interquartile range. Non-parametric tests of the median were conducted to calculate any significant differences between medians. A receiver operating characteristics (ROC) curve was also produced to determine the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the different growth times. The unpaired Student's *t*-test or Mann–Whitney *U*-test was used for the analysis of quantitative data.

During the study period, the BacT/Alert blood culture system was employed. Two pairs of blood culture vials were used for each patient, with a total recommended extracted volume of blood of 20–40 ml. These four vials, two for aerobic and two for anaerobic culture, were termed a 'blood culture batch'. Blood cultures were performed depending on medical criteria in patients with suspected sepsis; there is no predictive model able to identify which patients should have blood cultures done.

A blood culture was considered to be a true positive (bacteraemia) when at least one of the following microorganisms was isolated in at least one blood culture vial: Gram-negative bacilli, fungi, and Gram-positive cocci different from coagulase-negative Staphylococcus. A blood culture was also considered to be a true positive when coagulase-negative Staphylococcus was isolated in at least one vial of each pair and the patient presented a compatible clinical workup of bacteraemia. The blood culture was considered contaminated when coagulase-negative Staphylococcus, *Bacillus sp*, *Propionibacterium acnes*, or *Corynebacterium sp* was isolated in a single vial without any clinical indication. When coagulase-negative Staphylococcus isolated from a single vial was associated with intravascular catheter colonization (over 15 colony-forming units) caused by the same microorganism, the blood culture was considered positive if the patient's doctor initiated treatment based on this result. The growth time in hours was defined as the time elapsed between incubation and the detection of growth utilizing the automated blood culture system.

The same episode was considered bacteraemia if a new extraction with growth of the same microorganism was produced in the 5 days following the first extraction.

Patients were considered to have received prior antibiotic therapy if they received at least one dose of antibiotics in the 7 days prior to the blood culture. This information was analysed only in bacteraemic patients.

3. Results

During the study period from January 2011 to June 2013, 6816 blood culture batches were collected from patients older than 14 years of age. From this total, 1350 positive blood culture batches were associated with 1334 episodes of suspected bacteraemia; 590 of these corresponded to true bacteraemia and 744 were classified as contaminated blood culture batches.

3.1. Microorganisms

Of the 1334 blood culture batches with growth in the blood culture vials, true-positive bacteraemia ($n = 590$) was produced by enterobacteria (46.8%), coagulase-negative Staphylococcus (15.2%), *Staphylococcus aureus* (8.3%), polymicrobial infections (5.4%), *Enterococcus sp* (5.3%), non-fermenting Gram-negative bacilli (4.2%), *Streptococcus pneumoniae* (2.5%), *Candida sp* (2%), and *Streptococcus agalactiae* (1%).

Of the contaminated blood cultures ($n = 744$), coagulase-negative Staphylococcus made up 70.2%, *Propionibacterium sp* 9.5%, polymicrobial microorganisms 8.2%, and *Corynebacterium sp* 4.6%.

3.2. Growth times of contaminated and true-positive blood cultures

The growth time of the contaminated blood cultures ($n = 744$) was a median 20.6 h (IQR 17.04–32.16 h). Regarding the true-positive blood culture bacteraemia ($n = 590$), the growth time was a median 12.72 h (IQR 10.08–17.58 h). The difference between the two growth times was statistically significant ($p < 0.001$).

A ROC curve (Figure 1) was produced to determine the sensitivity, specificity, PPV, and NPV of the growth time as a determinant of contaminated growth. The area under the curve was 0.80, with a 95% confidence interval of 0.771–0.826.

In true bacteraemia, if the first vial detecting microorganism growth did so in less than 12 h, a specificity of over 95% and sensitivity of 45.3 % was found (PPV 87.8%, NPV 68.7%). In total, 90% of the contaminants grew beyond 14.7 h (sensitivity 90.5%, specificity 63.4%, PPV 65.9%, NPV 90.7%) and 95% of the contaminants grew beyond 12 h (sensitivity 95%, specificity 43.6%, PPV 57.8%, NPV 92.1%).

Comparing the growth times between distinct microorganisms producing true bacteraemia, there were microorganisms with significantly slower growth than others, as was the case with *Bacteroides sp* and *Candida sp* (Table 1) (Figures 2 and 3).

Comparing the growth times of coagulase-negative staphylococci in bacteraemia cases (90 cases; median 16.32 h, IQR 12.84–19.92 h) to the growth times of coagulase-negative staphylococci considered to be a contaminant (538 cases; median 19.20 h, IQR 16.80–24.72 h), the difference in growth times was found to be statistically significant with $p < 0.001$ (Figures 2 and 3).

Analyses of the growth times of true bacteraemia according to whether the patient received antibiotic therapy in the week prior

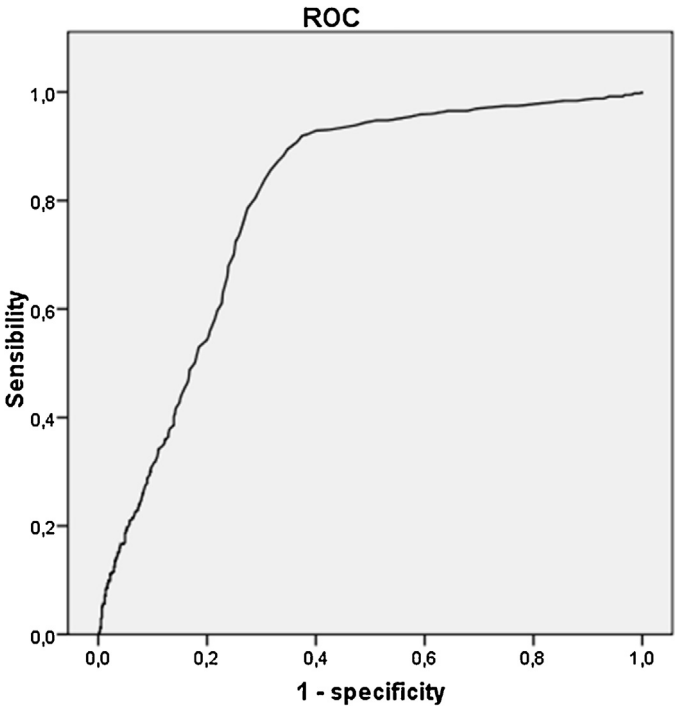

Figure 1. ROC curve (area under the curve 0.80, 95% confidence interval 0.771–0.826).

to culture were conducted using the 547 true bacteraemia cases for which this information was collected.

3.3. Bacteraemia without antibiotic therapy in the week prior to the event

In total, 419 patients with bacteraemia had not received antibiotic therapy in the week prior to blood culture collection. In 233 patients (55.6%), the microorganism responsible for the bacteraemia grew in less time than the median time for all bacteraemia (12.72 h of 590 bacteraemia). The median time to growth in the group of true bacteraemia from patients who had not received antibiotic therapy in the week prior to diagnosis was 12 h (IQR 9.84–15.96 h).

3.4. Bacteraemia with antibiotic therapy in the week prior to the event

In total, 128 patients with bacteraemia had received prior antibiotic therapy, and of these patients, 45 (35.2%) had cultures that grew in less time than the median time for all bacteraemia (12.72 h of 590 bacteraemia). The median in the group of true bacteraemia involving patients who had received antibiotic therapy was 14.16 h (IQR 11.28–20.40 h).

The administration of antibiotics in the week prior to acquiring bacteraemia was found to significantly slow and delay the growth time of microorganisms in blood culture vials ($p < 0.001$).

4. Discussion

Based on the results presented, the time at which microorganism growth is detected in blood culture vials using the automated

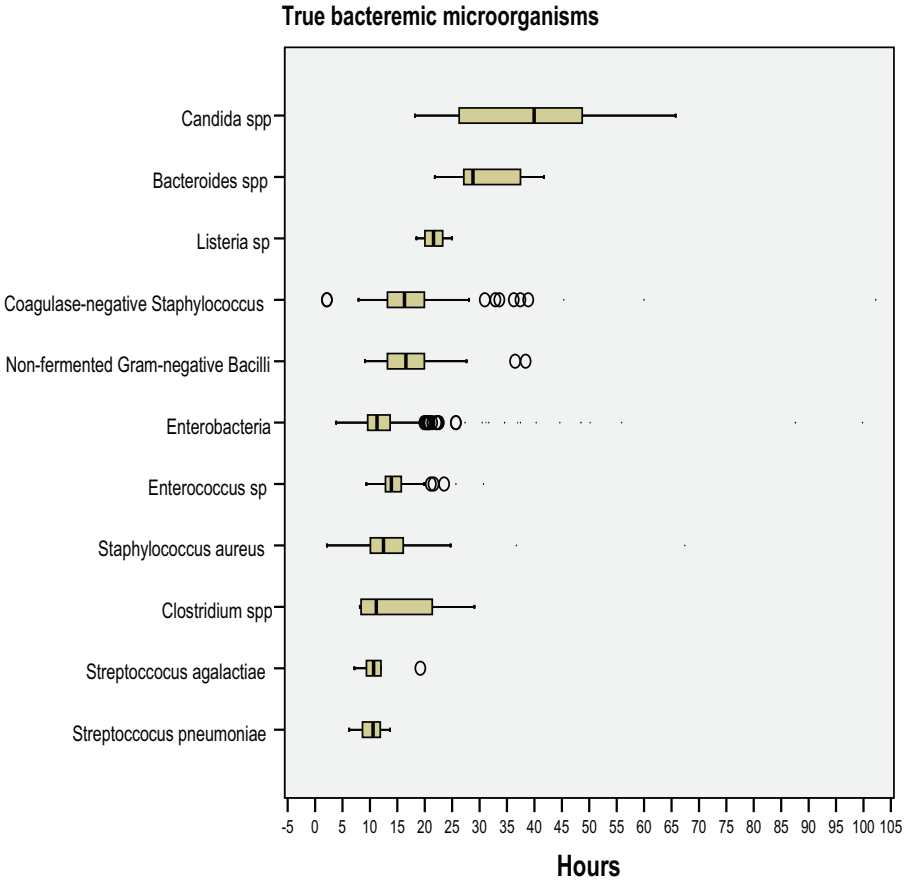

Figure 2. Growth times in hours of true bacteraemic microorganisms.

Table 1
Growth times in hours by quartiles of the principle microorganisms responsible for bacteraemia: 507 cases

Microorganism (cases: 507/590)	25%	Median	75%	*p*-Value (difference in growth times)
Enterobacteria (*n* = 276)	9.6	11.28	13.68	<0.001
Non-fermenting Gram-negative bacilli (*n* = 25)	12.84	16.92	19.32	Enterobacteria/non-fermented Gram-negative bacilli
Coagulase-negative Staphylococcus (*n* = 90)	13.2	16.32	19.92	<0.001
Enterococcus sp (*n* = 31)	12.72	13.92	15.84	Coagulase-negative Staphylococcus/all other Gram-positive cocci
Streptococcus pneumoniae (*n* = 15)	8.64	10.56	12	
Staphylococcus aureus (*n* = 49)	9.84	12.48	16.32	
Clostridium sp (*n* = 4)	8.28	11.16	25.2	
Bacteroides sp (*n* = 5)	24.48	28.8	39.6	<0.016
Candida sp (*n* = 12)	25.98	39.96	50.12	Bacteroides and Candida/all other microorganisms

BacT/Alert system could alert clinicians to the existence of true bacteraemia, with a high specificity at 95% and an important sensitivity of over 45.3 % if this growth is produced in the first 12 h after incubation.

With regard to this timescale, it must be considered that distinct types of microorganism, such as *Bacteroides sp* and *Candida sp*, have significantly slower growth times than other microorganisms.

Several investigations have described a correlation between high bacterial load and faster growth times. Thus, it has been suggested that growth time could be a marker of bacterial load.[8,9] Some researchers have found that the time it takes for a blood culture to provide a positive test result can help to indicate the origin of bacteraemia produced by *S. aureus*. If the patient has not received antibiotic treatment and the blood culture is obtained by venipuncture, a growth time of less than 14 h suggests the existence of an endovascular focus.[10,11] This fact is most likely related to the higher bacterial load in intravascular locations, which would explain faster culture growth times.

This pattern could explain why contaminated blood cultures have slower growth times than uncontaminated cultures – by the mere fact that the bacterial load is lower in contaminated blood cultures than in cultures from patients with an active infection. It is important to highlight that bacteria often found as contaminants are not necessarily non-pathogens. Currently all microorganisms may be a pathogenic agent and therefore cannot be ignored.

The prior administration of antibiotic therapy will also delay microorganism growth times in blood culture vials. The reason for the delay in growth could be the fact that prior antibiotic therapy reduces the bacterial load or even complicates the growth, inhibiting it completely in blood culture vials. To date, we have not been able to identify any reports in the literature confirming the data described or that quantify the delay in growth times if the patient has received prior antibiotic therapy.

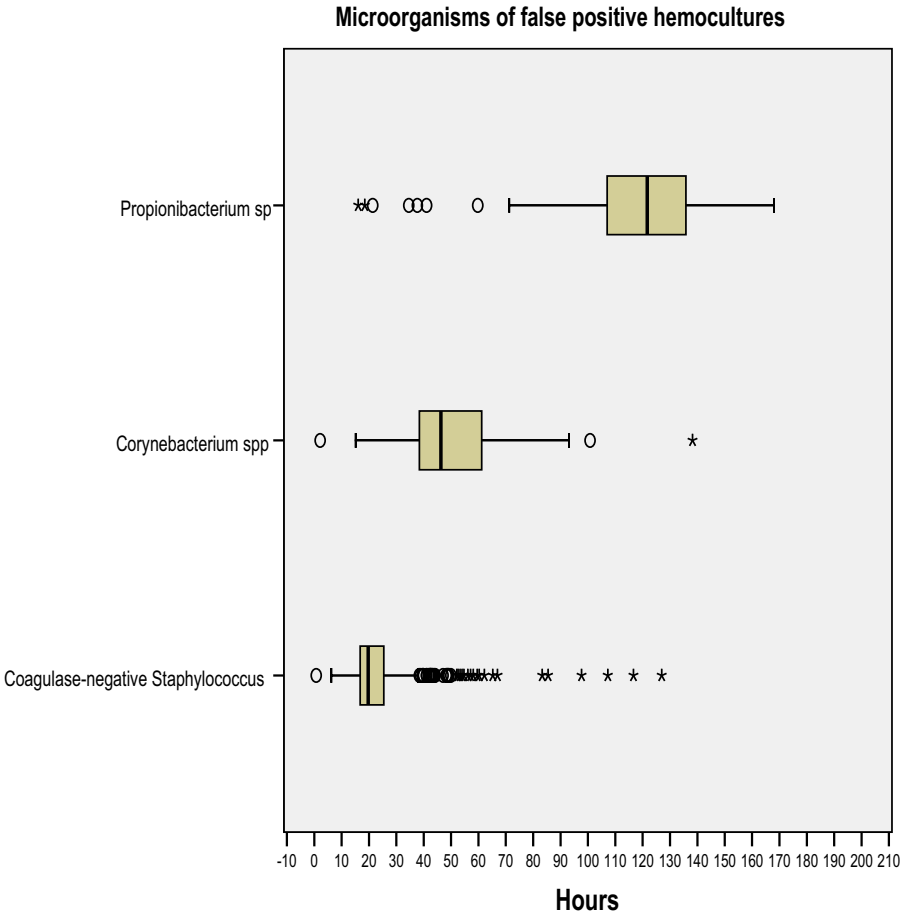

Figure 3. Growth times in hours of contaminant microorganisms.

A third factor that could influence the growth time results, but that was not analysed in this study, is the blood extraction volume per vial. The routine use of blood volumes of at least 30 ml is based on prior studies in which the authors have shown that the optimal volume of blood extraction for the diagnosis of bacteraemia is 20–30 ml.[12,13] The presence of an inadequate volume of blood in a culture vial could imply a lower bacterial load and longer growth times.

Knowledge of these growth times could inform the clinician of the existence of true bacteraemia several hours before the definitive identification of the microorganism responsible. At this time it would be sufficient to collect the times at which microorganism growth is detected in blood culture vials using the automated BacT/Alert system. In addition, this time to growth could help clinicians to distinguish bacteraemia from contamination due to coagulase-negative Staphylococcus, depending on the number of bottles in which the microorganism is detected.

The significant delay observed in the growth times of Candida is noteworthy. It is known that the yield of Candida in blood cultures is approximately 50–70%.[14] Mortality in the group of patients with invasive candidiasis is between 40% and 60%.[15,16] Various models and predictive scales for candidaemia have been attempted, but the fundamental utility of the majority of these models is the exclusion of candidaemia and not its diagnosis.[17–22] The delayed growth times of Candida together with insufficient harvesting of this microorganism for its diagnosis could suggest the need to confirm whether prolonged incubation times of blood cultures from patients suspected to have candidaemia could increase the value of these blood cultures given the significant delay that the growth times present.

There are limitations to these results due to the study has been performed at a single centre. It was not determined whether the volume of blood extracted was correct, although recommendation is at least 20 ml for each patient. Furthermore, the time between blood extraction and the beginning of the incubation period was not monitored, although this should be only of a few minutes. Also, this was a retrospective analysis. Prior antibiotic use was considered in the event that the patient received at least one dose of antibiotics during the week before blood extraction. The effect of this would probably differ depending on the number of antibiotic doses received, the number of days on which antibiotics were taken, and the time between the last antibiotic dose and blood extraction. Nevertheless, it seems important to consider the prior administration of antibiotics to try to optimize the extraction of blood cultures.

In conclusion, the time at which growth is detected in blood cultures using the automated BacT/Alert system could inform clinicians of whether the culture represents true bacteraemia or not, pending the time required for a definitive identification of the microorganism involved. The factors that influence these growth times are the association of the culture with true bacteraemia, the prior use of antibiotic therapy, and the type of microorganism. These results should be confirmed in other studies in different geographic areas.

Conflict of interest: The authors declare no conflicts of interest.

References

1. Baron EJ, Weinstein MP, Dunne WM, Yagupsky P, Welch DF, Wilson DM. Cumitech 1C, blood cultivate IV. Washington, DC: ASM Press; 2005.
2. Clinical and Laboratory Standards Institute. Principles and procedures for blood cultures. Approved guideline. CLSI document M47-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.
3. Cockerill III FR, Wilson JW, Vetter EA, Goodman KM, Torgerson CA, Harmsen WS, et al. Optimal testing parameters for blood cultures. *Clin Infect Dis* 2004;**38**:24–30.
4. Richter SS, Beekmann SE, Croco JL, Diekema DJ, Koontz FP, Pfaller MA, Doern GV. Minimizing the workup of blood culture contaminants: implementation and evaluation of a laboratory-based algorithm. *J Clin Microbiol* 2002;**40**:2437.
5. Gómez J, García-Vázquez E, Baños R, Canteras M, Ruiz J, Baños V, et al. Predictors of mortality in patients with methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia: the role of empiric antibiotic therapy. *Eur J Clin Microbiol Infect Dis* 2007;**26**:239–45.
6. Lombardi DP, Engleberg C. Anaerobic bacteremia: incidence, patient characteristics, and clinical significance. *Am J Med* 1992;**92**:53–60.
7. Arpi M, Renneberg J, Andersen HK, Nielsen B, Larsen SO. Bacteremia at a Danish university hospital during a twenty five-year period (1968–1992). *Scand J Infect Dis* 1995;**27**:245–51.
8. Rogers MS, Oppenheim BA. The use of continuous monitoring blood culture systems in the diagnosis of catheter related sepsis. *J Clin Pathol* 1998;**51**:635–7.
9. Marra AR, Edmond MB, Forbes BA, Wenzel RP, Bearman GM. Time to blood culture positivity as a predictor of clinical outcome of *Staphylococcus aureus* bloodstream infection. *J Clin Microbiol* 2006;**44**:1342–6.
10. Martinez JA, Pozo L, Almela M, Marco F, Soriano A, Lopez F, et al. Microbial and clinical determinants of time-to-positivity in patients with bacteraemia. *Clin Microbiol Infect* 2007;**13**:709–16.
11. Khatib R, Riederer K, Saeed S, Johnson LB, Fakih MG, Sharma M, et al. Time to positivity in *Staphylococcus aureus* bacteremia: possible correlation with the source and outcome of infection. *Clin Infect Dis* 2005;**41**:594–8.
12. Weinstein MP, Reller LB, Murphy JR, Lichtenstein KA. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. *Rev Infect Dis* 1983;**5**:35–53.
13. Ilstrup DM, Washington II JA. The importance of volume of blood cultured in the detection of bacteremia and fungemia. *Diagn Microbiol Infect Dis* 1983;**1**:107–10.
14. Clancy CJ, Nguyen MH. Finding the "missing 50%" of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. *Clin Infect Dis* 2013;**56**:1284–92.
15. Guery BP, Arendrup MC, Auzinger G, Azoulay E, Borges Ś M, Johnson EM, et al. Management of invasive candidiasis and candidemia in adult non-neutropenic intensive care unit patients: part I. Epidemiology and diagnosis. *Intensive Care Med* 2009;**35**:55–62.
16. Falagas ME, Apostolou KE, Pappas VD. Attributable mortality of candidemia: a systematic review of matched cohort and case–control studies. *Eur J Clin Microbiol Infect Dis* 2006;**25**:419–25.
17. Michalopoulos AS, Geroulanos S, Mentzelopoulos SD. Determinants of candidemia and candidemia-related death in cardiothoracic ICU patients. *Chest* 2003;**124**:2244–55.
18. Paphitou NI, Ostrosky-Zeichner L, Rex JH. Rules for identifying patients at increased risk for candidal infections in the surgical intensive care unit: approach to developing practical criteria for systematic use in antifungal prophylaxis trials. *Med Mycol* 2005;**43**:235–43.
19. Ostrosky-Zeichner L, Sable C, Sobel J, Alexander BD, Donowitz G, Kan V, et al. Multicenter retrospective development and validation of a clinical prediction rule for nosocomial invasive candidiasis in the intensive care setting. *Eur J Clin Microbiol Infect Dis* 2007;**26**:271–6.
20. Shorr AF, Sun X, Johannes RS, Yaitanes A, Tabak YP. Validation of a novel risk score for severity of illness in acute exacerbations of COPD. *Chest* 2011;**140**:1177–83.
21. León C, Ruiz-Santana S, Saavedra P, Almirante B, Nolla-Salas J, Alvarez-Lerma F, et al. EPCAN Study Group, A bedside scoring system ("Candida score") for early antifungal treatment in nonneutropenic critically ill patients with Candida colonization. *Crit Care Med* 2006;**34**:730–7.
22. Leroy G, Lambiotte F, Thévenin D, Lemaire C, Parmentier E, Devos P, Leroy O. Evaluation of "Candida score" in critically ill patients: a prospective, multicenter, observational, cohort study. *Ann Intensive Care* 2011;**1**:50.