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Rational empiric antibiotic therapy in clinical practice and policy making: uncertainties, probabilities, and ethics

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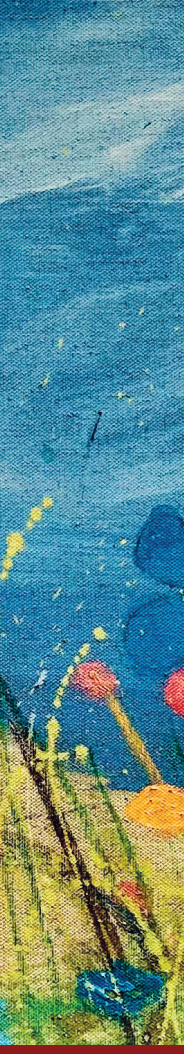

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Distribution and clinical determinants of Time to positivity of blood cultures in patients with neutropenia

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ABSTRACT

Objectives: Blood cultures (BC) are essential in the evaluation of neutropenic fever. Modern BC systems have significantly reduced the time to positivity (TTP) of BC. This study explores the probability of bacteraemia when BC have remained negative for different periods of time.

Methods: All adult patients with neutropenia and bacteraemia were included (January 2012–February 2016). Predictive clinical factors for short (≤16 hours) and long (>24 hours) TTP were determined. The residual probability of bacteraemia was estimated for the scenario of negative BC 24 hours after collection.

Results: The cohort consisted of 154 patients, accounting for 190 episodes of bacteraemia. Median age 61 years, 60.5% were male. In 123 (64.7%) episodes, BC yielded a single Gram-positive microorganism and in 49 (25.8%) a Gram-negative microorganism (median TTP 16.7, 14.5 hours respectively, $p<0.01$). TTP was ≤24 hours in 91.6% of episodes. Central line associated bacteraemia was associated with long TTP. The probability of bacteraemia if BC had remained negative for 24 hours, was 1-3%.

Conclusions: The expected TTP offers guidance in the management of patients with neutropenia and suspected bacteraemia. The knowledge of negative BC can support a change in working diagnosis, and impact clinical decisions as soon as 24 hours after BC collection.

INTRODUCTION

During neutropenia, patients are at high risk to develop bacterial bloodstream infections (BSIs), which are associated with substantial morbidity and mortality.[1,2] Prompt initiation of empiric antimicrobial therapy therefore is an essential part of the treatment of neutropenic patients with fever and is universally advocated by guidelines.[3-8]

However, fever does not necessarily indicate the presence of bacterial infection. Viral and fungal infections regularly occur in this patient population. Furthermore, non-infectious origins of fever, for example paraneoplastic, transfusion-related, medicinal or thromboembolic events, can generate symptoms that clinicians usually associate with BSI. It is notoriously difficult to distinguish bacterial from non-bacterial pathology based on the first clinical assessment.

Ruling-out bacteraemia plays an important part in excluding bacterial infection. In contrast to excluding localised infection, using for example radiographic examinations, blood cultures are time-consuming. Despite tremendous efforts, there is still no reliable alternative for blood cultures when it comes to the detection of bacteraemia.[9,10] However, due to improved microbiological techniques such as continuous monitoring systems, the time to positivity (TTP) of BCs has improved markedly over the past decades. At present, the majority of BCs becomes positive within 24 hours.[11-13]

In previous studies, data on patients with neutropenia are limited. The distribution of TTP in neutropenic patients may be different from the general population. Both the immunodeficiencies and the specific microbiology in these patients may influence TTP. Knowledge of TTP is particularly relevant with respect to the differential diagnosis of (persisting) fever and may have consequences for both the diagnostic approach and rational choice and duration of empiric antimicrobial therapy. Taking into account the negative implications of broad-spectrum empiric therapy like toxicity, interactions with co-medication, and development of antimicrobial resistance, timely differentiation between bacterial and non-bacterial pathology are warranted.[14-17]

The objective of this study was to determine the distribution of the time-to-positivity (TTP) of BCs in patients with neutropenia, that is to assess after how many hours of negative BC results detection of bacteraemia becomes unlikely. In addition, we aimed to identify clinical characteristics that predict late (>24 hours) positivity of BCs in this specific patient population.

METHODS

Setting and Study population

The study was performed at the Leiden University Medical Center (LUMC), a tertiary care hospital with a dedicated haematopoietic stem cell transplantation program. During the period of study (January 1st 2012 to February 1st 2016), all consecutive patients, aged ≥18 years with neutropenia and mono- or polymicrobial bacteraemia were included. Eligible patients were identified through search of the BC database of the Department of Medical Microbiology. Neutropenia was defined as an absolute neutrophil count below 0.5 x 10^9 cells/L at the day of BC collection. Patients were eligible for inclusion if the episode of bacteraemia developed during admission as well as when presenting at the emergency or an outpatient department. Multiple episodes of bacteraemia per patient were allowed if the antimicrobial therapy for the previous episode had been completed and clinical cure had been achieved.

Coagulase-negative staphylococci (CoNS) and other common skin contaminants needed to be isolated from at least two separate BC-sets, have identical susceptibility patterns and reason for initiation of directed antimicrobial therapy, to be eligible for inclusion.

In the LUMC, standard empiric antibiotic treatment in case of suspected sepsis during neutropenia is cefuroxime plus gentamicin or vancomycin plus ceftazidime, depending on antibiotic pre-treatment.

Data collection

Clinical variables were collected from the electronic patient records, and included patient demographics, medical history and clinical variables at the time BCs were obtained. Other measured parameters included admission to the intensive care, length of hospitalisation and 30-day mortality. The classification of the source of infection was based on the documented diagnosis and review of the available clinical, radiological and microbiological information. For central line- associated bloodstream infection (CLABSI) specifically, a central line had to be in place within the 48 hours prior to BC collection. The clinical data were independently collected and classified by two of the investigators (EW and ML). In case of inconsistencies, a third investigator (MB) was involved. Detailed data about the BCs were obtained from the database of the Department of Medical Microbiology. Approval for the study was obtained from the institutional ethical re-view board.BC handling procedures and laboratory techniques

A minimum of one BC set (anaerobic and aerobic culture bottle) was collected. The time of bedside collection was automatically recorded, as an imperative part of the BC ordering

procedure, in the electronic patient file. BCs were directly transported to the Department of Medical Microbiology and placed in the BACTEC FX continuous monitoring system (Becton Dickinson B.V., Breda), which detects microbiological growth through measurement of bacterial CO_2 production and automatically records the time of BC positivity.

Definition of time to positivity.

TTP was defined as the time between BC collection and the positive BACTEC signal. If multiple separate BCs from one patient were collected within a time frame of two hours, the bottle with the shortest TTP was used for analysis.

Because of its design as a 'real-life' clinical study, laboratory closing hours had to be taken into account. When a BC was incubated after working hours (Monday to Friday after 5 p.m., Saturday/Sunday after 1.00 p.m.) technical registration in the culture system was delayed to the next morning. In bottles that reached positivity before registration, the culture was recorded positive at the time of registration, between 8 and 9 a.m., instead of upon positive signalling. This resulted in an overestimation of the TTP. In unregistered bottles that were placed after 17:00, referred to as 'evening bottles', the TTP is ≤16 hours by definition (in weekends TTP ≤20 hours).

Statistical analyses

Normally and non-normally distributed continuous variables were compared by Student-*t* test and Mann-Whitney U test, respectively. Univariate risk factor analysis for binominal variables was performed using cross tables and Fisher's exact statistical tests. Results are reported as risk ratios (RR) with 95% confidence intervals (95%CI). multivariable analysis was performed to analyse independent predictors for short (≤16 hours) and late (>24 hours) TTP. The variables for the multivariable analysis were selected based on $p < 0.20$ in the univariate analysis and plausibility.

To detect a potential effect of a repeated measurement phenomenon through inclusion of >1 episode for a proportion of the patient population, both the univariate and multivariable analyses were repeated using a generalized estimating equation model correcting for repeated measures. The median TTP was calculated by including the overestimated TTP of 'evening' and 'weekend bottles', and in an extra analysis with bottles with exact TTP data only (Supplementary files). 'Weekend bottles' were excluded from the categorical TTP analyses, as it was unknown whether TTP was ≤16 hours in these episodes.

The association between short TTP (≤16 hours) and 30-day mortality was evaluated by Kaplan-Meier analysis, using the Log-rank test.

All statistical analyses were performed with the IBM SPSS Statistics, version 23.

RESULTS

Study population characteristics

A total of 190 episodes in 154 patients were included. The median age was 61 years (IQR 47-67) and 115 (60.5%) were men. In the majority (66.8%) of patients, neutropenia was caused by the antineoplastic treatment of a haematological malignancy. Antimicrobial agents, mainly consisting of ciprofloxacin (93 episodes) and penicillin (77 episodes) prophylaxis, were used in 75.8% of patients at the time of BC collection (Table 1). Hundred and twenty-six patients (81.8%) had one episode of bacteraemia, 25 patients (16.2%) had two episodes, and three patients (1.9%) were included with three episodes. One patient had five separate episodes of bacteraemia. In 20 episodes (10.2%) BCs were placed and became positive during evening hours ('evening bottles', TTP≤16 hours) and in 8 episodes (4.2%) during a weekend ('weekend bottles', TTP≤20 hours).

In Table 2 the pathogen distribution and median TTP per micro-organism are summarized. In the majority of episodes the source of bacteraemia was chemotherapy induced mucositis or a CLABSI was diagnosed.

Time-to-positivity

Overall, the median TTP was 15.6 hours (IQR 13.6-18.9 hours). Figure 1 displays the distribution of TTP. The TTP was ≤24 hours in 91.6 % of episodes. In all episodes without antibiotic pre-treatment, the TTP was less than 24 hours. The median TTP was shorter in episodes with Gram-negative bacteraemia, as compared to episodes with Gram-positive bacteraemia (14.5 hours versus 16.7 hours respectively, p<0.01).

Short time to positivity (≤16 hours)

In the univariate analysis, presentation at the outpatient clinic or Emergency Department, being clinically moderately or severely ill and a gastro-intestinal source of infection correlated with short TTP (≤16 hours). Mono-microbial Gram-negative bacteraemia and polymicrobial bacteraemia were associated with TTP ≤16 hours (Table 3). In the multivariable analysis (Supplementary files) presentation at the outpatient clinic or first aid department (adjusted OR 3.53 95%CI 1.14-10.90, p<0.03), making a moderately or severely ill impression during physical examination (adjusted OR 2.51, 95%CI 1.19-5.32, p=0.02), a gastro-intestinal tract infection (adjusted OR 2.25 95% CI 1.09-4.65, p=0.03) and Gram-negative bacteraemia (adjusted OR 2.82 95%CI 1.07-7.45, p=0.04) were independently associated with a TTP ≤16 hours. A history of diabetes (adjusted OR 0.23 95%CI 0.07-0.74, p=0.01) was inversely correlated with a short TTP. No relevant effect of including >1 episode per patient was detected through correction by a generalized estimating equations model.

Table 1. Characteristics of bacteraemia in patients (n=154, 190 episodes) with neutropenia.

Clinical variable	n = 190 (100%)
Patient demographics	
Male gender	115 (60.5)
Age (years) , (median IQR)	61 (47-67)
Medical history	
Haematological malignancy	127 (66.8)
Solid malignancy	26 (13.7)
Stem cell transplantation	75 (39.5)
Solid transplantation	2 (1.1)
Diabetes mellitus	21 (11.1)
Prednisone use past 6 months	122 (64.2)
Clinical presentation	
Temperature>38.0 °C	155 (81.6)
Systolic blood pressure (mmHg) (median IQR)	122 (109-138)
Pulse rate (bpm) (median IQR)	106 (91-120)
Neurologic status:	
No abnormalities	122 (64.2)
Somnolent	7 (3.7)
Confused	9 (4.7)
Sedated	7 (3.7)
Clinical impression:	
Acutely or moderately ill	83 (43.7)
Not ill	63 (33.2)
Central venous catheter in situ	126 (66.7)
Antibiotic pre-treatment	144 (75.8)
Hospitalization before BC[a] (days) (median IQR)	12 (0.1-18.4)
Microbiological parameters	
Monomicrobial Gram-positive bacteraemia:	123 (64.7)
Monomicrobial Gram-negative bacteraemia	49 (25.8)
Polymicrobial bacteraemia	18 (9.5)
Source of infection	
Gastro-intestinal	67 (35.3)
Central venous catheter	56 (29.5)
Respiratory	12 (6.3)
Endovascular (e.g. thrombus)	7 (3.7)
Urinary tract	5 (2.6)
Skin and soft tissue	5 (2.6)
Other	6 (3.2)
Not identified	32 (16.8)
Outcome	
ICU/MCU[b] admission during hospitalization	48 (25.3)
Hospitalization after BC (days) (median IQR)	14 (9-25)
30-day mortality	47 (24.7)
Time between culture and death (days) (median IQR)	58 (12-193)

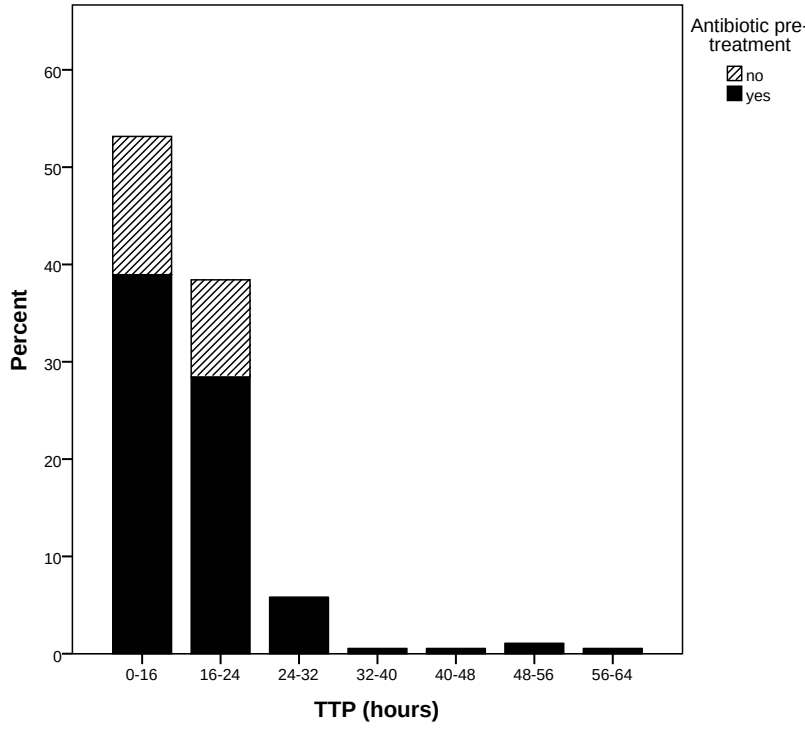
Legend: [a] BC=blood culture [b] ICU/MCU = intensive care unit / medium care unit. [c] In-hospital mortality = mortality during hospitalization episode.

Table 2. Pathogen distribution and median time to positivity(TTP) (190 episodes).

Pathogen	Number of episodes (%)	Median TTP (hours) (range)
Overall	190 (100)	15.6 (7.3-62.5)
Gram-positive:	123 (65)	16.7 (8.5-62.5)
Enterococcus spp.	57 (30)	15.5 (10.5-52.0)
Streptococcus spp.	19 (10)	15.0 (8.5-47.4)
Staphylococcus aureus	4 (2)	15.5 (13.0-19.8)
CoNS[a]	36 (19)	19.1 (12.0-31.2)
Other (including anaerobes)	7 (4)	19.3 (9.3-51.7)
Gram-negative:	49 (26)	14.5 (7.3-55.6)
Escherichia coli	24 (13)	12.5 (7.3-18.3)
Pseudomonas aeruginosa	11 (6)	17.8 (14.5-23.4)
Enterobacter spp.	5 (3)	14.4 (11.8-16.3)
Klebsiella spp.	3 (2)	13.2 (12.9-14.6)
Other (including anaerobes)	6 (3)	22.0 (13.0-55.6)
Polymicrobial	18 (9)	14.9 (8.5-22.0)

Legend: [a] CoNS = *Coagulase-negative Staphylococcus spp.*

Figure 1. Time to positivity and antibiotic pre-treatment distribution (190 episodes)



Long time to positivity (>24 hours)

Univariate analysis demonstrated that antibiotic pre-treatment, CoNS bacteraemia and CLABSI were associated with a TTP >24 hours. An acutely or moderately ill impression on physical examination was inversely correlated with long TTP (RR 0.33, 95%CI 0.11-0.99, p=0.05) (Table 3).

In the multivariable analysis, CLABSI (adjusted OR 4.66, 95%CI 1.41-15.41, p=0.01) was an independent predictor of a long TTP (Supplementary files). No relevant effect of including >1 episode per patient was detected through correction by a generalized estimating equations model.

In 16 (8.4%) episodes the TTP was above 24 hours (Table 4). The BCs in the group of patients with TTP>24 hours were mono-microbial and contained exclusively Gram-positive or anaerobic microorganisms. In 8 (50%) episodes a CLABSI with CoNS was diagnosed. No bacteraemia or sepsis attributable 30-day mortality occurred in the patients with a TTP>24 hours. There was no association between 30-day mortality and TTP<16 hours (p = 0.33)

Table 3. Univariate analysis characteristics in 182 episodes, for time to positivity (TTP) ≤16 hours and TTP>24 hours.

Characteristic	Short TTP (TTP≤16 hours)		Long TTP (TTP>24 hours)	
	RR[a] (95% CI)	P-value	RR (95% CI)	p-value[b]
Patient demographics				
Male gender	1.07 (0.81-1.39)	0.65	0.83 (0.32-2.16)	0.79
Age > 60 years	0.96 (0.74-1.25)	1.00	1.29 (0.50-3.30)	0.79
Medical history				
Haematological malignancy	0.98 (0.76-1.27)	1.00	1.06 (0.38-2.90)	1.00
Solid malignancy	1.11 (0.78-1.59)	0.66	1.60 (0.49-5.18)	0.43
Stem cell transplantation	0.99 (0.77-1.30)	1.00	0.88 (0.33-2.30)	1.00
Diabetes mellitus	0.70 (0.40-1.21)	0.16	0.54 (0.08-3.87)	1.00
Prednisone use past 6 months	0.88 (0.68-1.14)	0.35	0.53 (0.21-1.34)	0.18
Clinical presentation				
Temperature>38.0 °C	1.19 (0.80-1.75)	0.43	N.A[d]	0.08
Systolic blood pressure <100 mmHg	1.28 (0.95-1.73)	0.20	N.A.	0.13
Pulse rate >100 bpm	1.00 (0.76-1.31)	1.00	2.03 (0.68-6.13)	0.28
Neurologic symptoms	1.03 (0.65-1.64)	1.00	0.65 (0.09-4.60)	1.00
Acutely or moderately ill clinical presentation	1.65 (1.18-2.31)	<0.01	0.32 (0.11-0.99)	0.05
Central venous catheter in situ	0.74 (0.57-0.95)	0.04	3.3 (0.77-13.93)	0.10
Antibiotic pre-treatment	0.82 (0.62-1.08)	0.22	N.A.	0.03
Outpatient department	1.50 (1.18-1.91)	<0.01	0.20 (0.03-1.45)	0.08
Microbiological parameters				
Monomicrobial Gram-positive bacteraemia	0.65 (0.51-0.84)	<0.01	2.47 (0.73-8.34)	0.18
Monomicrobial CoNS[c] bacteraemia	0.34 (0.18-0.63)	<0.01	3.92 (1.58-9.75)	<0.01
Monomicrobial Gram-negative bacteraemia Anaerobic bacteraemia	1.48 (1.16-1.89)	<0.01	N.A.	<0.01
Polymicrobial bacteraemia	1.47 (1.10-1.95)	0.05	N.A.	0.37
Source of infection				
Gastro-intestinal	1.37 (1.07-1.77)	0.02	0.65 (0.17-1.92)	0.58
Central venous catheter	0.56 (0.38-0.81)	<0.01	4.95 (1.80-13.58)	<0.01
Respiratory tract	0.74 (0.37-1.46)	0.38	N.A.	0.60
Endovascular (e.g. thrombus)	0.77 (0.32-1.82)	0.70	N.A.	1.00
Urinary tract	N.A.	0.07	N.A.	1.00
Skin and soft tissue	N.A.	0.13	N.A.	1.00
Other	1.20 (0.68-2.16)	0.69	N.A.	1.00
Not identified	0.34 (0.05-2.46)	0.69	0.34 (0.05-2.46)	0.48

Legend: [a] RR = relative risk. [b] P-values were calculated using the Fishers exact test [c] CoNS = Coagulase-negative *Staphylococcus spp* test [d] N.A.: Relative risk not available as one of the cells contained a zero.

Table 4. Characteristics of episodes with a time to positivity >24 hour.

TTP (hours)	Sex, age (years)[a]	Patho-gen[b]	haematological malignancy or stem cell transplant	Solid malig-nancy	Adequate empiric treatment[c]	Source of infection	30-day mortality, cause of death
24.4	M, 65	STAPHA	yes	no	yes	CLABSI[d]	treatment withdrawal[e]
24.5	M, 65	STAPHA	yes	no	yes	CLABSI	no
24.6	M,36	ENCOFE	yes	no	yes	unknown	No
25.2	F, 73	STAPHA	yes	no	no	CLABSI	cerebral vascular infarct
26.0	F, 70	STAPHA	yes	no	no	CLABSI	no
26.1	M, 59	CLOSIN	no	no	no	gastro-intestinal	no
26.2	F, 65	ENCOFE	yes	no	yes	gastro-intestinal	no
26.4	M, 51	STAPHA	yes	no	no	CLABSI	no
26.5	F, 51	STAPEP	yes	no	no	CLABSI	fungal infection
28.5	F, 61	STAPEP	yes	no	yes	CLABSI	no
31.2	M, 32	STAPEP	yes	no	no	CLABSI	no
34.6	M, 69	FUSO	yes	no	yes	CLABSI	no
47.4	F, 69	STREOR	no	yes	yes	gastro-intestinal	treatment withdrawal
51.7	M, 27	ROMUCI	yes	no	yes	CLABSI	no
55.6	F, 50	FUSO	yes	yes	yes	gastro-intestinal	treatment withdrawal
62.5	M, 64	ENCOFA	yes	yes	no	CLABSI	treatment withdrawal

Legend: [a] M = male, F = female. [b] STAPHA = *Staphylococcus haemolyticus*, CLOSIN = *Clostridium innocuum*, ENCOFE = E*nterococcus faecium*, STAPEP = *Staphylococcus epidermidis*, FUSO = *Fusobacterium spp.*, STREOR = *Streptococcus oralis*, ROMUCI = *Rothia mucilaginosa*, ENCOFA = *Enterococcus faecalis*. [c] Adequate empirical therapy based on in vitro susceptibility testing. [d] CLABSI = central line associated bloodstream infection. [e] Treatment withdrawal = Discontinuation of treatment of the underlying disease/malignancy.

DISCUSSION

Main findings

For clinical practice, the most relevant finding of the present study is that in the vast majority (91.6%) of patients with neutropenia and bacteraemia, BC TTP is ≤24 hours, in particular in patients without antibiotic pre-treatment (100%). All patients with Gram-negative aerobic bacteraemia had positive BC results within 24 hours after venepuncture.

Moreover, from the data this study provides on TTP during neutropenia, the probability of positive cultures 24 hours after venepuncture can be estimated by using both the proportion of cultures with late positivity (8.4%) and the proportion of bacteraemia among all BCs that are obtained in suspected sepsis in this population (Box 1). The latter is highly dependent on the patient population, e.g. haematological versus oncological, and varies, based on previous reports, between 15% and 29%.[18-23] This corresponds with a general probability of approximately 1-3% of a positive BC when cultures are still negative after 24 hours.

BOX 1. Formula for calculating the probability of a positive blood culture (BC) after 24 hours of incubation.

A tool for translation to clinical practice

$$P = \frac{(1-TTPe) * X}{1-(TTPe * X)} * 100\%$$

P = Probability of a positive BC when the BC has remained negative after 24 hours of incubation.

X = The fraction of positive BCs among all BCs that are obtained in suspected sepsis in patients with neutropenia (centre-specific).

TTP_e = proportion of positive BCs that are positive in ≤24 hours = 0.92 (95% CI 0.88-0.96) For a more conservative estimation of P, the lower bound of the 95 % CI (0.88) can be chosen for TTPe.

Example

In a medical centre an estimated 20% of BCs in patients with neutropenia and fever is positive.

Then (use formula, X = 0.2 TTP_e = 0.92): If BCs are still negative after 24 hours of incubation, the probability that the culture will yet become positive is approximately 2 %.

Alternatively, using the lower bound of the 95 % CI (0.88) results in a probability of approximately 3 %.

Comparison with other studies

Only few studies have reported on TTP of BCs in patients with neutropenia, and most have focused on one specific pathogen. In these studies the correlation between TTP and clinical characteristics were only partially addressed. In the largest cohort study to date by Martinez *et al*. (n=134), TTP was 11.7 hours (IQR 9-17 hours).[11] The median TTP found in the current study was relatively longer than in previous studies.[11,12] This can be explained by the difference in the definition of TTP. In our study, TTP was measured starting from the time of BC collection at the bedside. In contrast, in most studies TTP was defined as the time between incubation and the positive signal. For interpretation and discussing the clinical consequences, the complete process should be taken into account.

We found that Gram-positive bacteraemia was relatively more common in patients with neutropenia, compared to the general patient population.[11,13] This could be explained by both the relatively high incidence of intravascular line infections in our patient population and the use of chemoprophylaxis (ciprofloxacin). This shift from Gram-negative to Gram-positive organisms in patients with neutropenia during the past few decades, due to chemo-prophylaxis, has been reported previously.[24]

Predictors for short and long TTP

The data show an association between the physicians' clinical assessment at presentation (i.e. a more or less subjective measure) and TTP, TTP being shorter when the patient was assessed to be clinically ill. This could be explained by the fact that patients with

Gram-negative bacteraemia usually are more severely ill compared to patients with bacteraemia caused by CoNS and *Enterococcus* spp., which have longer TTP. Other biological factors like circulating lipopolysaccharides (LPS) during Gram-negative BSI may contribute to this mechanism. The association was less evident for the more objective parameters, such as blood pressure and pulse rate, underlining the additive value of bedside clinical evaluation.

Strengths and limitations

A shortcoming of the present study is the unknown exact TTP in the patients with 'evening and weekend' culture bottles. The inclusion of these patients, in which the actual TTP was shorter than the registered TTP, results in an overestimation of actual TTP. A separate analysis after exclusion of these episodes showed that this barely influenced overall TTP results (Supplementary files). The adjusted definition of TTP, representing the 'practical TTP' instead of the 'microbiological TTP' used in previous studies, could be considered a limitation. However, the practical definition was chosen for reasons of applicability to daily clinical practice, as clinicians are generally not precisely informed about transportation times and details on incubator placing. Transportation and communication logistics differ between hospitals which may impair the applicability of our results to centres where BCs are not directly transported to the laboratory and placed in the incubator or positive BCs are not directly communicated to the attending physician. However, direct transportation, incubation and communication represent best practice in the field of infectious diseases and clinical microbiology.[25]

The present study has several other strengths. First, to the best of our knowledge it represents the largest cohort of patients with neutropenia, providing insight in the distribution of TTP. Secondly the design of the study enables translation to daily clinical practice. Furthermore, the collection of clinical data in addition to microbiological data provides additional insight into the distribution of TTP in subcategories of patients.

Implications for practice and future research

Knowledge on the low probability of bacteraemia after 24 hours is valuable for the management of patients with fever and neutropenia. Primarily, in the absence of a source of infection, a preliminary negative BC, should impel to (re)investigate other (non-bacterial) causes of fever.

In addition, in the scenario of a confirmed source of infection (e.g. pneumonia), preliminary negative BC results can be of value in the early de-escalation of antimicrobial therapy towards a targeted –small spectrum- treatment.

In conclusion, when using modern BC systems and adequate logistics, the probability of bacteraemia when BCs are negative after 24 hours is very low. Based on the data of the present study, there is no rationale to postpone investigations into an alternative diagnosis beyond this point in time.

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REFERENCES

1. Bastug A, Kayaaslan B, Kazancioglu S, But A, Aslaner H, Akinci E, Yetkin MA, Kanyilmaz D, Eren SS, Bodur H. Emergence of multidrug resistant isolates and mortality predictors in patients with solid tumours or haematological malignancies. J Infect Dev Ctries 2015;29:1100-7.
2. Lyman GH, Michels SL, Reynolds MW, Barron R, Tomic KS, Yu J. Risk of mortality in patients with cancer who experience febrile neutropenia. Cancer 2010;116:5555-63
3. Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA, Raad II, Rolston KV, Young JA, Wingard JR. Clinical Practice Guideline for the Use of Antimicrobial Agents in Neutropenic Patients with Cancer: 2010 Update by the Infectious Diseases Society of America. Clin Inf Dis 2011;52:56-93.
4. Klastersky J, de Naurois J, Rolston K, Rapoport B, Maschmeyer G, Aapro M, Herrstedt J. Management of febrile neutropenia: ESMO Clinical Practice Guidelines. Ann Oncol 2016;27:111–18.
5. Averbuch D, Orasch C, Cordonnier C, Livermore DM, Mikulska M, Viscoli C, Gyssens IC, Kern WV, Klysova G, Marchetti O, Engelhard D, Akova M. European guidelines for empirical antibacterial therapy for febrile neutropenic patients in the era of growing resistance: summary of the 2011 4th European Conference on Infections in Leukemia. Haematologica 2013;98:1826-35.
6. Philips R, Hancock B, Graham J, Bromham N, Jin H, Berendse S. Prevention and management of neutropenic sepsis in patients with cancer: summary of NICE guidance 2012;345:e5368.
7. Link H, Böhme A, Cornely OA, Höffken K, Kellner O, Kern WV, Mahlberg R, Maschmeyer G, Nowrousian MR, Ostermann H, Ruhnke M, Sezer O, Schiel X, Wilhelm M, Auner HW. Antimicrobial therapy of unexplained fever in neutropenic patients. Ann Hematol 2003;82:105–17.
8. SWAB guidelines for Antibacterial therapy of adult patients with Sepsis. Dutch Working Party on Antibiotic Policy (SWAB). 2010.
9. Suberviola B, Marquez-Lopez A, Castellanos-Ortega A, Fernandez-Mazarrasa C, Santibanez M, Martinez LM. Microbiological diagnosis of sepsis: polymerase chain reaction system versus blood cultures. American Journal of Critical Care. 2016;25(1):68–75
10. Korpelainen S, Intke C, Hämäläinen S, Jantunen W, Pulkki K. Soluble CD14 as a Diagnostic and Prognostic Biomarker in Hematological Patients with Febrile Neutropenia. Dis Markers. 2017
11. Martínez JA, Pozo L, Almela M, Marco F, Soriano A, López F, Balasso V, Aguilar J, Mensa J. Microbial and clinical determinants of time-to-positivity in patients with bacteraemia. Clin Microbiol Infect. 2007;13:709-16.
12. Pardo J, Klinker KP, Borgert SJ, Trikha G, Rand KH, Ramphal R. Time to positivity of blood cultures supports antibiotic de-escalation at 48 hours. Ann Pharmacother. 2014;48:33-40.
13. Lambregts MMC, Bernards SM, van der Beek M, Visser LG, de Boer MGJ. Distribution and clinical predictors of time to positivity (TTP) of blood cultures (BC): tools for a shorter duration of empirical antimicrobial therapy for suspected sepsis . IDweek; 2016: poster session; 1867.
14. Alp S, Akova M. Management of febrile neutropenia in the era of bacterial resistance. Ther Adv Infect Dis 2013;1:37-43.
15. Gudiol C, Carratalà J. Antibiotic resistance in cancer patients. Expert Rev Anti Infect Ther. 2014;12:1003-16.
16. Biedenbach DJ, Moet GJ, Jones RN. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program. Diagn Microbiol Infect Dis 2004; 50:59-69.

17. Macesic N, Morrissey CO, Cheng AC, Spencer A, Peleg AY. Changing microbial epidemiology in hematopoietic stem cell transplant recipients: increasing resistance over a 9-year period. Transpl Infect Dis. 2014;16:887-96.
18. Slobbe L, Waal L, Jongman LR, Lugtenburg PJ, Rijnders BJ. Three-day treatment with imipenem for unexplained fever during prolonged neutropaenia in haematology patients receiving fluoroquinolone and fluconazole prophylaxis: a prospective observational safety study. Eur J Cancer. 2009;45:2810-7.
19. Zinner SH. Changing epidemiology of infections in patients with neutropenia and cancer: emphasis on gram-positive and resistant bacteria. Clin Infect Dis 1999;29:490-4.
20. Winston DJ, Lazarus HM, Beveridge RA, Hathorn JW, Gucalp R, Ramphal R, Chow AW, Ho WG, Horn R, Feld R, Louie TJ, Territo MC, Blumer JL, Tack KJ. Randomized, double-blind, multicenter trial comparing clinafloxacin with imipenem as empirical monotherapy for febrile granulocytopenic patients. Clin Infect Dis 2001;32:381-90.
21. Feld R, DePauw B, Berman S, Keating A, Ho W. Meropenem versus ceftazidime in the treatment of cancer patients with febrile neutropenia: a randomized, double-blind trial. J Clin Oncol. 2000;18:3690-8.
22. Klastersky J, Ameye L, Maertens J, Georgala A, Muanza F, Aoun M, Ferrant A, Rapoport B, Rolston K, Paesmans M. Bacteraemia in febrile neutropenic cancer patients. Int J Antimicrob Agents 2007;30:51-9.
23. Viscoli C, Cometta A, Kern WV, Bock R, Paesmans M, Crokaert F, Glauser MP, Calandra T. Piperacillin-tazobactam monotherapy in high-risk febrile and neutropenic cancer patients. Clin Microbiol Infect. 2006;12:212-6.
24. Ramphal R. Changes in the Etiology of Bacteraemia in Febrile Neutropenic Patients and the Susceptibilities of the Currently Isolated Pathogens. Clin Infect Dis 2004;39:S25-31.
25. Idelevich EA, Seifert H, Sundqvist M, Scudeller L, Carmeli Y, Becker K. Microbiological diagnostics of bloodstream infections in Europe – an ESGBIS Survey. 27th European Congress of Clinical Microbiology and Infectious Diseases. 2017; Oral session:OS0747.

SUPPLEMENTARY DATA

Multivariable logistic regression

Table S1A. Multivariable logistic regression for short time-to-positivity (<16 hours).

Variables in the equation	B	S.E.	Wald	df	Sig.	Exp(B)	95% CI
Moderately/acutely ill	0.92	0.38	5.78	1	0.02	2.51	1.19-5.32
Central line	0.70	0.56	1.57	1	0.21	2.04	0.67-5.96
Presentation at the outpatient department	1.26	0.58	4.79	1	0.03	3.53	1.14-10.90
Source: gastro-intestinal	0.81	0.37	4.84	1	0.03	2.25	1.09-4.65
Diabetes mellitus	-1.49	0.60	6.10	1	0.01	0.23	0.67-0.64
Systolic blood pressure <100 mmHg	.043	0.54	0.01	1	0.94	1.04	0.36-3.00
Gram-negative bacteraemia	1.04	0.50	4.37	1	0.04	2.82	1.07-7.45
Constant	-1.25	0.60	4.38	1	0.04	0.29	

Table S1B. Multivariable logistic regression for long time-to-positivity (>24 hours

Variables in the equation	B	S.E.	Wald	df	Sig.	Exp(B)	95% CI
Gram-positive bacteraemia	0.31	0.72	0.19	1	0.67	1.36	0.33-5.57
Clinical impression: not ill	0.65	0.56	1.34	1	0.25	1.92	0.64-5.76
CLABSI[a]	1.54	0.61	6.34	1	0.01	4.66	1.41-15.41
Constant	3.58	0.66	29.70	1	0.00	0.03	

Legend: [a] CLABSI = central line associated bloodstream infection

Separate analysis of time-to-positivity (TTP) after exclusion of evening/ weekend bottles

Table S2. Pathogen distribution and median time-to-positivity (TTP) after exclusion of weekend and evening bottles

Pathogen	Number of episodes (%)	Median TTP (hours) (range)
Overall	162 (100)	15.9 (7.3-62.48)
Gram-positive:	105 (65)	16.8 (9.3-62.5)
Enterococcus spp.	45 (28)	15.5 (10.5-62.5)
Streptococcus spp.	15 (9)	14.8 (11.1-47.4)
Staphylococcus aureus	4 (2)	15.5 (13.0-19.8)
CoNS[a]	34 (21)	19.3 (12.0-31.2)
Other (including anaerobes)	7 (4)	19.3 (9.3-51.7)
Gram-negative:	42 (26)	14.5 (7.3-55.6)
Escherichia coli	18 (11)	12.1 (7.3-15.3)
Pseudomonas aeruginosa	11 (7)	17.8 (14.5-23.4)
Enterobacter spp.	4 (2)	13.8 (11.8-16.3)
Klebsiella spp.	3 (2)	13.2 (12.9-14.6)
Other (including anaerobes)	6 (4)	22.1 (13.0-55.6)
Polymicrobial	15 (9)	15.0 (10.15-22.0)

Legend: [a] CoNS = *Coagulase-negative Staphylococcus spp.*