Pediatric RESEARCH

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QUALITY IMPROVEMENT ARTICLE

Optimized blood culture strategy to document febrile neutropenia

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BACKGROUND: Poor and delayed microbiological documentation of episodes of febrile neutropenia (EFN) deserves improvement. We assessed the impact of a new blood culture (BC) sampling protocol to optimize the diagnosis of bloodstream infection during EFN, compared with standard of care protocol.

METHODS: This pre/post intervention included patients who presented an EFN in a pediatric hematology-oncology center. Data were compared between 1-year periods P1 (110 EFN, 53 patients) and P2 (124 EFN, 53 patients). Pre-intervention settings were 1−2 mL of blood cultured per BC set and several samplings over days (multisampling strategy) during period P1 vs. one unique early sampling of a large volume of blood (0.5–60 mL) depending on patient weight during period P2 (single-sampling weight-adapted strategy). Microbial detection and time-to-diagnosis were evaluated.

RESULTS: Seventeen EFNs were microbiologically documented in P1 (15.5%) and 26 in P2 (21%). The rate of positive BC sets increased during P2 (10.4% vs. 5.8%). All cases of bacteremia were documented by BC drawn during the first 4 days of fever, and during P2 by samples obtained on the first day of fever.

CONCLUSIONS: Bacteremia detection was improved. This proof-of-concept study shows benefits of combining the single-sampling strategy with large weight-adapted blood sampling strategy during EFN.

Pediatric Research (2021) 89:1109–1116; https://doi.org/10.1038/s41390-020-1086-0

IMPACT:

- Combination of single-sampling and weight-adapted blood culture strategies showed benefits in the documentation of bloodstream infections during febrile neutropenia.
- Bacteremia detection was improved in this preliminary study and this warrants further evaluation in the overall pediatric population.
- We observed no adverse effects associated with the new strategy while overall blood sparing was improved and handling of intravascular devices was reduced.
- The good tolerance of the blood sampling suggests that the recommended 1% volume limitation in children could be reconsidered.
- A similar evaluation is justified in the overall pediatric population suspected for bloodstream infection.

INTRODUCTION

Fever with neutropenia is the most common complication in the treatment of childhood cancer.[1] When documented, 63% of episodes of febrile neutropenia (EFN) have a bacterial etiology, of which 75% are bacteremia.[2] The low frequency of microbiological documentation (21–70%) deserves to be optimized.[3,4] The first-line examination is blood culture (BC), and in both children and adults, the volume of blood cultured is the main determinant of BC yield.[5–8] In children, the volume adapted on the patient's age or, more practically, on the patient's weight significantly improves bacteremia detection.[5–8] The study of Kellogg et al. in which the increase in the volume of blood was achieved by increasing either the blood volume within a bottle (from 0.5 to 10 mL) or the total number of bottles collected through the number of samples (from 1 to 3)[6] prompted several microbiology societies to issue guidelines for the optimal volume to be cultured based on the child's weight.[9–11] As the single-sampling strategy of a large blood volume was previously shown to positively impact the BC sensitivity in adult patients,[7,12,13] this strategy warrants consideration in the neutropenic pediatric population considering that single sampling is usual in children.

We describe a single-center pre-/post-intervention study that evaluates the impact of a change in blood culturing process on the detection of bacteremia in children with febrile neutropenia.

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This work was presented (poster) at the 38th RICAI Congress in Paris (16 and 17 December 2018).

Received: 14 May 2020 Revised: 10 July 2020 Accepted: 10 July 2020
Published online: 28 July 2020

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The intervention is culturing one large, weight-based volume of blood on the day of fever onset rather than repeated smaller volumes of blood collected over days that was standard of care. As there are no data currently available on such an intervention strategy in children, this study was conducted as a proof-of-concept in a population highly susceptible to bloodstream infections (BSI).

MATERIALS AND METHODS

Patients and data collection

The study was conducted in the pediatric hematology−oncology unit (18 hospitalization beds, 700 annual inpatient admissions) of the University Hospital of Montpellier, a large French regional center. All patients who presented an EFN and had BC were included. The study has been approved by the Research Ethics Committee of our institution under reference 2017_CLER-MTP_07–26. This observational study fell within routine practice with nonadditional procedures applied to the patient so that the Ethics Advisory Committee waived the requirement to obtain a signed informed consent document, as accepted for research procedures involving no risk for the patient.

The following data were collected and comparatively analyzed for the pre- and post-intervention periods: patient age and weight at hospital admission, cancer diagnosis, treatment protocol, type of central venous catheter (tunneled external or totally implanted), condition of the insertion site, catheter removal, duration of neutropenia and fever, and duration and type of antibiotic treatment, number of BC sets drawn during the EFN, number and types of bottles collected, and BC results.

Definitions

Fever in a neutropenic child was defined as oral or rectal temperature ≥38.3 °C for more than 1 h. Neutropenia was defined as a neutrophil count below 500/mm^3. Febrile episodes were categorized into two groups, i.e., high risk and low risk of infection, based on expected duration of chemotherapy-induced neutropenia as defined in Table 1. A new EFN was defined when recurrent or recrudescent fever occurred after an initial resolution with antimicrobial therapy in a neutropenic patient

Table 1. Characteristics of febrile neutropenic episodes and blood cultures.

Variable	Episodes of febrile neutropenia (EFN)			Blood culture sets		
	Period 1 ($n = 110$ EFN)	Period 2 ($n = 124$ EFN)	*P* value	Period 1 No. (%) ($n = 431$ BC sets)	Period 2 No. (%) ($n = 336$ BC sets)	*P* value
Diagnosis, No. (%)						
Hematologic malignancies	65 (59.1%)	89 (71.8%)	0.07			
Solid tumors	43 (39.1%)	31 (25.0%)				
Others	2 (1.8%)	4 (3.2%)				
Risk of infection, No. (%)[a]						
High	82 (74.5%)	105 (84.7%)	0.05			
Low	28 (25.5%)	19 (15.3%)				
Type of central venous catheter, No. (%)						
Tunneled external	61 (55.5%)	102 (82.3%)	<0.001			
Totally implanted	49 (44.5%)	22 (17.5%)				
Condition of the insertion site, No. (%)						
Inflammation	17 (18.1%)	20 (18.0%)	>0.9			
No inflammation	77 (81.9%)	91 (82.0%)				
BC results, No. (%)						
Positive	17 (15.5%)	26 (21.0%)	0.3	25 (5.8%)	35 (10.4%)	0.02
Negative	93 (84.5%)	98 (79.0%)		406 (94.2%)	301 (89.6%)	
Microorganisms, No. (%)						
Gram-positive	7 (41.2%)	11 (42.3%)	0.7	9 (36.0%)	20 (57.2%)	0.3
Gram-negative	8 (47.0%)	13 (50.0%)		13 (52.0%)	13 (37.1%)	
Polymicrobial	2 (11.8%)	2 (7.7%)		3 (12.0%)	2 (5.7%)	
Fever duration, median (IQR), days	2 (1–4)	2 (1–5)	0.1			
Neutropenia duration, median (IQR), days	11 (6–21)	11 (6–23)	0.8			
Antimicrobial therapy duration, median (IQR), days	9 (5–17)	11 (7–25)	0.08			
Catheter removal after first day of fever						
Number	6	13	0.2			
Mean time (±SD), days	8 (±5.5)	6.4 (±3.6)	0.5			
Hospitalization length, median (IQR), days	13.5 (6–31)	20.5 (8–46)	0.08			

IQR interquartile range, *SD* standard deviation.
[a]The two groups were defined as follows: (i) high risk of infection when the expected duration of neutropenia was ≥7 days, which corresponds mainly to treatment protocols for acute lymphoblastic leukemia (ALL) (high risk or very high risk), ALL relapse, acute myeloid leukemia, graft conditioning, metastatic neuroblastoma, Burkitt and Ewing protocols, (ii) low risk of infection when the expected duration of neutropenia was <7 days corresponding to any other protocols.

who had at least two negative BC and was afebrile for at least 7 days.

A BC set refers to one blood specimen inoculated into one to several BC bottles. Blood specimens were exclusively collected from the central venous catheter, according to our institutional guidelines that favor venous capital sparing and painless procedures.[14] The aerobic and anaerobic bottles were incubated for 5 and 7 days, respectively. Microbiological definition of a possible BC contaminant included bacteria that belong to skin microbiota recovered from one bottle only.[14]

Study design

Depending on the period, 1−3 BC sets were collected in the first 24 h of fever; then, one BC set was sampled per day in case of persisting fever, irrespective of neutropenia resolution, in order to detect fungemia.[9] Additional collections were drawn in the presence of signs of sepsis at the physician's discretion. Following a change in BC sampling institutional guidelines in 2012, study design included a retrospective cohort study prior to the change (period 1, from November 2010 to October 2011) and a prospective cohort study (period 2, from May 2013 to April 2014) after the change.

During period 1, a BC set consisted of one single BacT/ALERT® PF aerobic pediatric culture bottle (bioMérieux, Marcy L'Etoile, France), seeded with 1−2 mL of blood drawn by syringe. No more than three BC sets were collected during the first 24 h of fever. During period 2, the volume of blood was based on patient weight and was drawn once (hereafter referred to as a single-sampling strategy). A BC set consisted of one to several BacT/ALERT®FA (aerobic) and BacT/ALERT®FN (anaerobic) bottles. A total of 1−6 bottles were each seeded with 3−10 mL of blood drawn by syringe to obtain a minimum total volume based on weight (Fig. 1), in line with Kellogg et al.'s proposals.[6] Fluid from flushing the catheter was also cultured. The impact of the intervention was assessed by comparing rates of documented EFN, time at which the index positive BC was sampled, the number of BC collected and the number of positive BC, and blood loss estimation and related adverse effects.

Patient management

Patients with EFN were managed based on institutional guidelines consistent with international recommendations.[1] In the absence of orientation, empirical broad-spectrum antibiotic therapy was initiated within 3 h of hospitalization; central venous catheter removal was left to physician's discretion depending on standard criteria,[15] and no change in this management occurred between the two periods. Vancomycin was added in case of grade 3 or 4 mucositis, alteration of the skin barrier, and/or when fever persisted for >48 h. Imipenem associated with an empirical antifungal treatment was administered when fever persisted for >5 days. Treatment was continued until the neutropenia resolved.

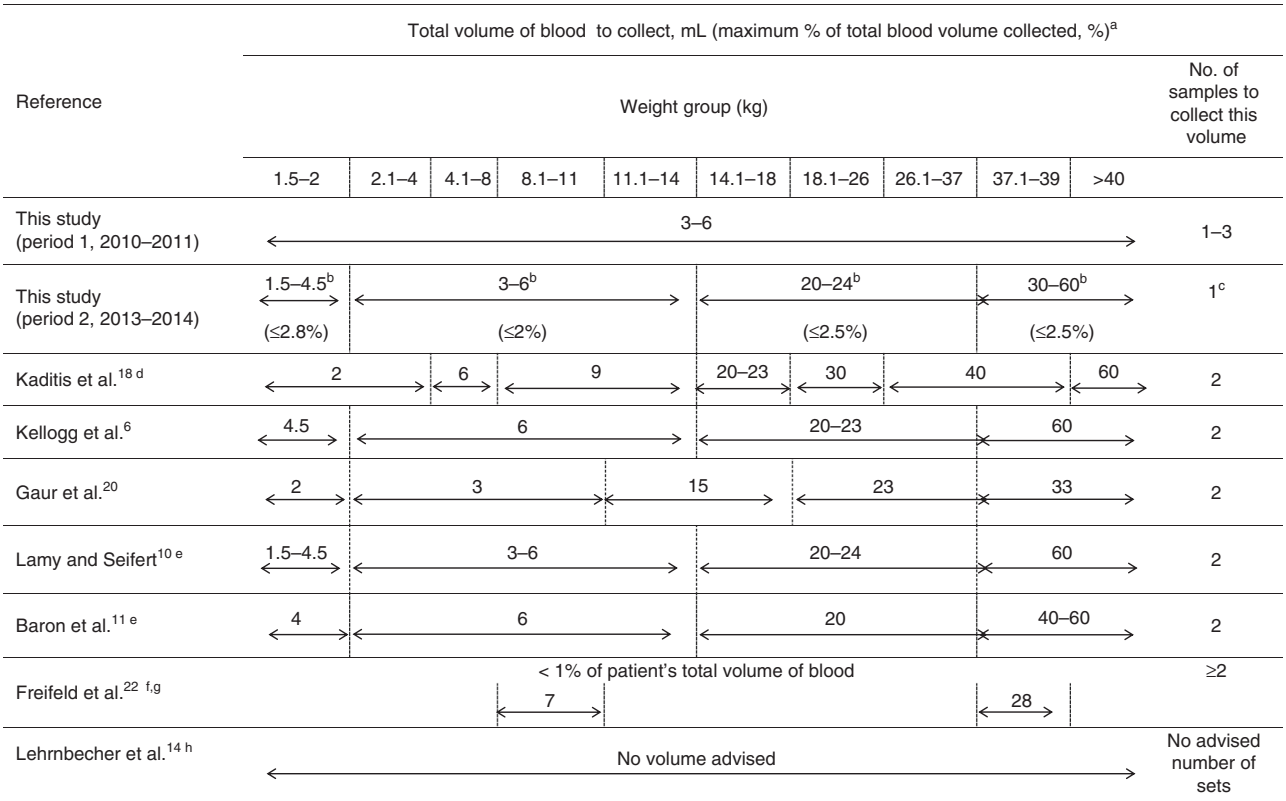

Fig. 1 Estimated volume of blood collected during the 1st 24 hours of EFN according to weight. This study, previous studies and advised volume to collect according to existing guidelines. The maximum % of total blood volume collected is presented for study period 2. The maximum % of total blood volume collected is presented for study period 2. **a** Rates estimated on the basis of 80−90 mL/kg for neonates, 70 mL/kg for children weighing ~10 kg, 60 mL/kg for patient weighing < 40 kg. **b** Volume adjusted to the weight, i.e. within a weight category, the heavier the child, the larger the volume. **c** Flushing fluid was also sampled (during the same sampling). **d** This study is the only published study that compared weight-adapted blood volumes to standard practice in pediatric oncology. **e**Recommendations from the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), and the Infectious Diseases Society of America (IDSA) with the American Society for Microbiology (ASM). **f** Guidelines for the management of EFN in patient with cancer or hematologic malignancies, Infectious Diseases Society of America (IDSA). **g** Volume presented as an example in guidelines.[22] **h** Guidelines for the management of EFN in children with cancer and hematopoietic stem-cell transplantation recipients, American Society of Clinical Oncology (ASCO).

Table 2. Characteristics of the population.

Variables	Period 1 No. patients (%) ($n = 53$ patients)	Period 2 No. patients (%) ($n = 53$ patients)	*P* value
Male sex	25 (47.2%)	34 (64.2%)	0.08
Age, mean (±SD), years	8.2 (±5.9)	9.1 (±6.3)	0.5
Weight, kg			
1.5–2	0	0	0.5
2.1–13	11	12	
13.1–37	25	19	
≥37.1	17	22	
Diagnosis			0.4
Malignant hemopathies	30 (56.6%)	33 (62.3%)	
ALL	23 (43.4%)	15 (28.3%)	
AML	2 (3.8%)	7 (13.2%)	
JMML	1 (1.9%)	0 (0%)	
Lymphomas	4 (7.6%)	11 (20.8%)	
Solid tumors	21 (39.6%)	16 (30.2%)	
Brain tumors	6 (11.3%)	0 (0%)	
Neuroblastoma	3 (5.7%)	7 (13.2%)	
Sarcoma	10 (18.9%)	7 (13.2%)	
Nephroblastoma	0 (0%)	1 (1.9%)	
Hepatoblastoma	0 (0%)	1 (1.9%)	
Histiocytosis	1 (1.9%)	0 (0%)	
Retinoblastoma	1 (1.9%)	0 (0%)	
Others	2 (3.8%)	4 (7.6%)	
Idiopathic medullary aplasia	2 (3.8%)	3 (5.7%)	
Beta-thalassemia	0	1 (1.9%)	
Number of EFN			
Mean per patient (±SD)	2.1 (±1.43)	2.2 (±1.40)	0.5

ALL acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *JMML* juvenile myelomonocytic leukemia.

Statistical analysis

Statistical analyses were performed using SAS Enterprise Guide 4.3 software (SAS Institute, Cary, NC). For quantitative variables, comparisons and analysis of the two groups were performed with either a Student's *t* test or a Wilcoxon−Mann−Whitney test where appropriate. For qualitative variables, comparisons and analysis of the two groups were conducted either with a chi-square test or a Fisher exact test where appropriate. A P value ≤ 0.05 was considered to reflect significance.

RESULTS

Patient, episode, and BC characteristics

A total of 234 EFN of 106 patients were included (1–7 EFN per patient). Of these, 110 EFN were recorded from 53 patients during period 1 (1–6 EFN per patient) and 124 EFN from 53 patients during period 2 (1–7 EFN per patient). Patient characteristics were comparable in both periods (Table 2). Characteristics of the EFN showed a trend of a greater number of hematologic malignancies during period 2 ($P = 0.07$), with a likely related increase in the number of external catheters ($P < 0.001$) (Table 1). A total of three patients exhibited a new EFN, 8, 13, and 24 days after resolution of a previous EFN, respectively.

A total of 767 BC sets (431 in period 1 and 336 in period 2) was collected during these 234 EFNs. During period 2, a higher rate of BC sets was sampled in patients with high risk of infection, hematologic malignancy, or externalized catheters (Table 1). Additionally, less additional BC samples were collected during period 2 in the days following the onset of fever (186 vs 281, $P < 0.01$) (Fig. 2a). During period 2, fewer BC sets per episode were sampled (2.71 vs. 3.92, $P = 0.02$) and fewer BC sets per episode were negative (2.43 vs. 3.39, $P < 0.01$).

Microbiologically documented EFN according to the strategy for taking BCs

A total of 43/234 EFN (18.4%) had positive BCs. Among them, nine could correspond to potential contamination when microbiological definition was strictly applied and five had an isolated positive flushing fluid culture (Table 3). These EFN were however considered to be microbiologically documented by the clinicians, and all patients received antimicrobial regimens toward the cultivated microorganism. Therefore, all EFN with positive BC were retained in the further analyses. The number of EFNs that were documented did not significantly differ between periods despite a trend (15.5% vs. 21.0%, $P = 0.3$), but more BC sets were positive in period 2 (10.4% vs. 5.8%, $P = 0.02$) (Fig. 2a). Characteristics of patients with documented EFN were comparable between periods. The only clinical factor with a significant correlation with culture positivity and the documentation of EFN was a high risk of infection. The number of positive bottles according to the study period is presented in Fig. 2b. Regardless of the period, all index positive BC were collected within the first 4 days of fever (Fig. 2c). During period 2, all but one episodes were documented by BC sets sampled at day 1 of fever, and the only positive episode at day 2 had no BC collected on day 1. Among the documented episodes,

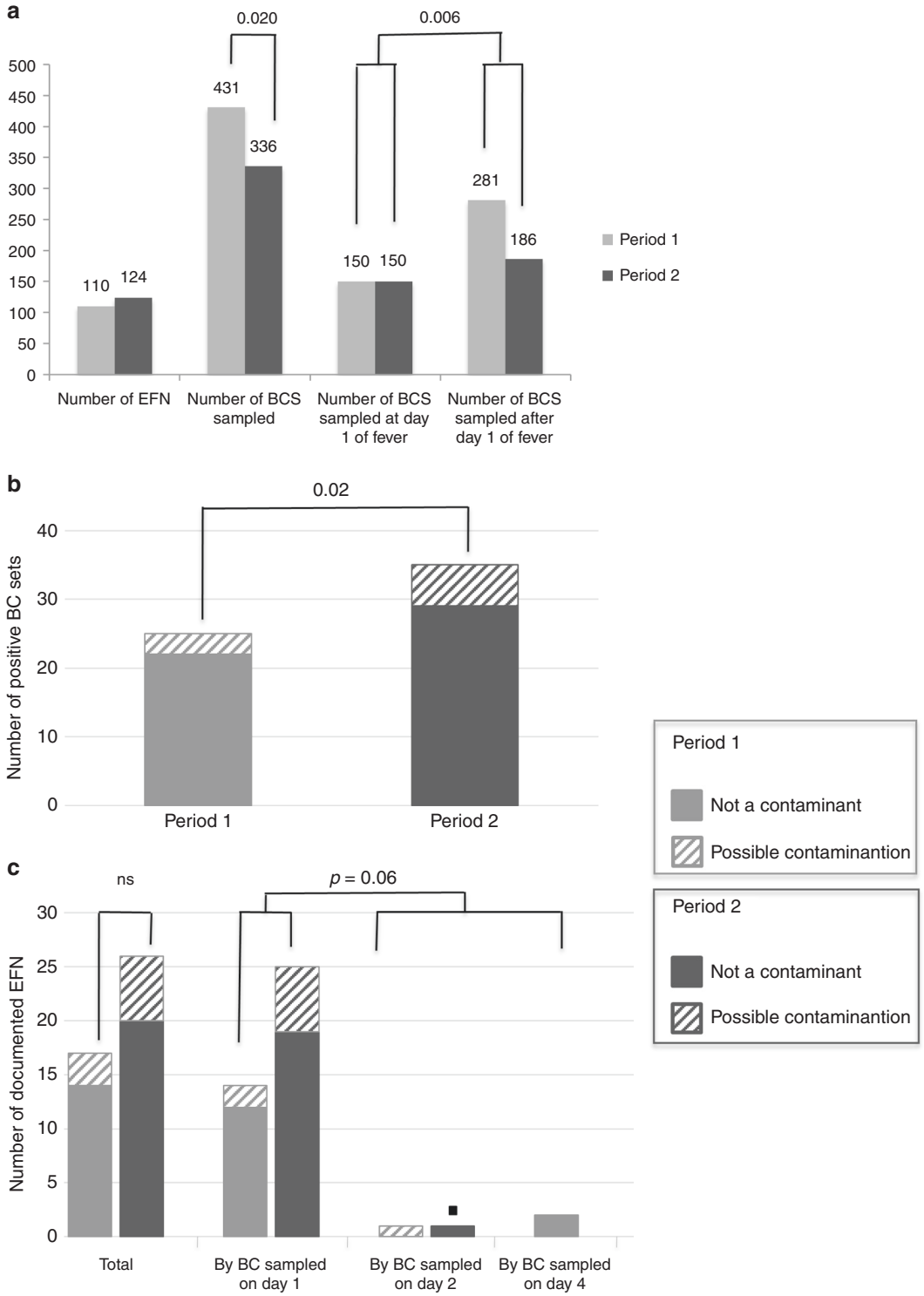

Fig. 2 Positive blood culture (BC) sets and documented episodes of febrile neutropenia (EFN) among all EFN and BC sets sampled during the two study periods. a Number of EFN and of BC sets sampled during the two study periods. **b** Number of positive BC sets during the two study periods and relative part of indeterminate result, i.e., possible contamination according to microbiological definition given in the text but considered as documenting the EFN and subjected to specific antimicrobial regimen by the clinicians (shaded). **c** Number of EFN documented in the two study periods according to the BC sampling day of fever, and relative part of indeterminate results as defined in (**b**) (shaded). In period 1, the EFN documented by BC sets sampled on day 2 of fever involved a coagulase negative *Staphylococcus* (EFN 13 in patient 10 in Table 3), and the two EFN documented by BC sets sampled on day 4 of fever involved an extended-spectrum beta-lactamase (ESBL)-producing *E. coli* (EFN 1 and 2 in the same patient 1 in Table 3). In period 2, the EFN documented by BC sets sampled on day 2 of fever involved a *Pseudomonas aeruginosa* (EFN 23 in patient 19 in Table 3 with no BC set sampled on day 1 of fever, indicated with a square), and the patient was not sampled for BC on day 1. n.s. not statistically significant. BCS blood culture sets, *P* values are indicated above the bars when <0.05.

Table 3. Microbiological and sampling data for EFN with positive blood culture sets during the two study periods.

Microorganism	Period 1					Period 2				
	EFN ID	Patient ID	No. of BC sets (No. of bottles) sampled during EFN (*n*)	No. of positive BC sets (*n*)	No. of positive bottles/total No. of bottles of the positive BC set (*n*/*n*)	EFN ID	Patient ID	No. of BC sets (No. of bottles) sampled during EFN (*n*)	No. of positive BC sets (*n*)	No. of positive bottles/total bottles of the positive BC set (*n*/*n*) ± positive flushing fluid (F)[a]
Gram-negative										
Escherichia coli	1	**1**[b]	10 (10)	2	1/1	18	14	6 (26 + 6F)	1	3/6 + F
	2	**1**[b]	2 (2)	1	1/1	19	15	3 (14 + 3F)	1	1/6
	–	–	–	–	–	20	**16**	4 (19 + 4F)	2	6/6 + F; 6/6 + F
	–	–	–	–	–	**21**	17	2 (12 + 2F)	1	1/6 + F
Klebsiella pneumoniae	3	**2**	4 (4)	1	1/1	22	**18**	2 (8 + 2F)	1	4/4 + F
	4	**3**[c]	7 (7)	1	1/1	–	–	–	–	–
	5	**3**[c]	7 (7)	1	1/1	–	–	–	–	–
Pseudomonas aeruginosa	6	4	2 (2)	1	1/1	23	19	8 (36 + 8F)	1	2/4 + F
	–	–	–	–	–	24	**20**	3 (3 + 3F)	1	1/1 + F
	–	–	–	–	–	25	21	6 (36 + 6F)	1	2/6
Enterobacter cloacae	7	2	6 (6)	1	1/1	–	–	–	–	–
Roseomonas mucosa	8	5	7 (7)	5	1/1	–	–	–	–	–
Acinetobacter sp.	–	–	–	–	–	26	22	2 (8 + 2F)	1	0/4 + F
Campylobacter jejuni	–	–	–	–	–	27	23	2 (12 + 2F)	1	4/6
Stenotrophomonas maltophilia	–	–	–	–	–	28	24	2 (3 + 2F)	1	2/2 + F
Neisseria sp.	–	–	–	–	–	29	**20**	2 (2 + 2F)	1	0/1 + F[d]
Gram-positive										
Streptococcus mitis/oralis	9	6	12 (13)	1	1/2	30	**18**	10 (46 + 10F)	3	6/6 + F; 2/4; 4/4 + F
	10	7	5 (5)	1	1/1	31	25	3 (18 + 3F)	1	6/6 + F
	–	–	–	–	–	32	26	6 (30 + 6F)	3	4/4 + F; 4/4; 6/6
	–	–	–	–	–	33	27	15 (60 + 15F)	2	4/4 + F; 4/4 + F
	–	–	–	–	–	34	28	1 (4 + 1F)	1	0/4 + F
Streptococcus sanguinis	–	–	–	–	–	35	29	1 (6 + 1F)	1	3/6 + F
Enterococcus faecalis	–	–	–	–	–	36	30	2 (12 + 2F)	2	0/6 + F; 2/6
Staphylococcus aureus	11	8	4 (4)	2	1/1	–	–	–	–	–
Staphylococcus epidermidis	–	–	–	–	–	**21**	17	2 (12 + 2F)	1	2/6
	–	–	–	–	–	37[e]	**31**	3 (12 + 3F)	1*	1/4[d]
	–	–	–	–	–	38[e]	**31**	3 (12 + 3F)	1*	1/4[d]
Staphylococcus hominis	–	–	–	–	–	39	32	3 (6 + 3F)	1*	0/1 + F
Unidentified CNS	12	9	2 (2)	2	1/1	40	33	3 (7 + 2F)	1*	0/1 + F
	13	10	4 (4)	1*	1/1	–	–	–	–	–
	14	11	1 (1)	1*	1/1	–	–	–	–	–
	15	12	13 (13)	1*	1/1	–	–	–	–	–
Micrococcus luteus	–	–	–	–	–	41	34	2 (8 + 2F)	1*	1/4
	–	–	–	–	–	42	35	1 (4 + 1F)	1*	1/4
Polymicrobial										
Streptococcus pneumoniae & Haemophilus influenzae	16	13	3 (3)	1	1/1	–	–	–	–	–
S. mitis/oralis & Candida albicans	17	**2**	7 (7)	2	1/1	–	–	–	–	–
E. coli & Granulicatella adiacens	–	–	–	–	–	43	**16**	5 (30 + 5F)	2	3/6 + F; 3/6
Total	17[f]	13	–	25	–	26[g]	23	–	35	–

Bold type indicates patients with multiple EFN with positive BC set or EFN with BC sets growing different species.
– indicates no episode with the corresponding bacteria.
EFN episode of febrile neutropenia, *BC* blood culture, *F* flushing liquid.
*Indicates a possible contamination (according to microbiological definition given in the text) that was considered by the clinicians as documenting the EFN and for antimicrobial treatment.
[a]For period 2, details for each positive BC set are given as follows: number of positive bottles/total bottle number of the BC set excluding the bottle containing the flushing fluid. Positivity of the bottle containing the flushing fluid is indicated by "+ F". Data for each positive BC set are indicated separated by a semicolon.
[b]EFN 1 and 2 occurred 13 days apart in patient 1 with severe neutropenic enterocolitis and septic shock. Catheter was not removed.
[c]EFN 4 and 5 occurred 24 days apart in patient 3, central venous catheter was not removed, inflammation at the insertion site was noted during the second EFN.
[d]Indicates the 3 EFN (EFN 29, 37, and 38) documented by a positive anaerobic blood culture bottle only.
[e]EFN 37 and 38 occurred 8 days apart in patient 31, inflammation was noted at the catheter insertion site at the time of the 2 EFN. The central venous catheter was not removed.
[f]In period 1, the patient weight distribution for the 17 EFN with positive BCs was as follows: 2–13 kg, 2 EFN; 13.1–37 kg, 7 EFN and ≥37 kg, 8 EFN.
[g]In period 2, the patient weight distribution for the 26 EFN with positive BCs was as follows: 2–13 kg, 6 EFN; 13.1–37 kg, 11 EFN and ≥37 kg, 9 EFN.

more were documented from BC sampled on day 1 of fever during period 2 ($P = 0.06$, Fig. 2c).

Epidemiology of BSI associated with EFN

Regardless of the period, bacteria recovered from BC were equally distributed between Gram-positive (52.1%) and Gram-negative (45.8%) bacteria. The most common microorganisms were coagulase negative staphylococci (CNS) (ten EFN, five with a methicillin-resistant isolate), *viridans* streptococci (nine EFN), *Escherichia coli* (seven EFN, two extended-spectrum beta-lactamase-producing isolates), *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (four episodes each, two multidrug-resistant *P. aeruginosa*). One EFN involved a methicillin-susceptible *Staphylococcus aureus* (Table 3). In period 2, five EFN were exclusively documented by the flushing fluid and three EFN were documented with one positive anaerobic bottle only (Table 3).

DISCUSSION

Optimal BC collection in children is still unclear with regard to the volume of blood to be collected.[8] Optimizing BC both in a timely and sensitive manner grows in importance with the increase in antibiotic resistance, especially in pediatric hematology-oncology.[16,17] We proposed a rational approach that combines a weight-based, graduated blood volume collection,[6,10,11] and the advantages of the single-sampling strategy.

This preliminary study aimed to assess the feasibility and the potential diagnostic value of culturing one large weight-based volume of blood in young hematology-oncology patients. Hence, the design and the size limit an in-depth analysis. Limitations include: (i) a difference in the number of patients at high risk of infection between periods, and small sample size that increased the chance of committing a type II error and hampered adjustment for confounders; and (ii) a comparator that was suboptimal in terms of BC sampling despite it corresponded to the standard of care of a large regional hematology-oncology center. Finally, period 1 was retrospective, which may have lowered the data completeness. Besides, some open questions include: (i) the conundrum of the clinical interpretation of potential contaminant microorganisms in patients with EFN;[14] and (ii) the consistency of catheter flushing fluid analysis. Overall, these limitations preclude from asserting that the intervention alone accounted for the higher rate of BC positivity found in period 2 vs. period 1. Despite these limitations, our results show the potential of combining a weight-based large volume of blood with the single-sampling strategy. Major improvements are a higher yield of recovered microorganisms notably for low level bacteremia, and an earlier microbial documentation of EFN through the increase of BC positivity rate for samples obtained without delay (day 1 of fever).

Weight-adapted volume of blood

Culturing a large volume of blood adapted to the child's weight increases the detection of BSI of low concentration,[5,6,18,19] takes into account that the microbial density is inversely proportional to the age,[5,6,20,21] and limits blood loss. The current guidelines from international societies for microbiology recommend 1−3 samplings of 1−2 bottles each, per episode,[9–11,22] but international groups managing pediatric patients with hematologic malignancies have so far neither adopted these guidelines nor recommended explicit weight-adapted volumes but an unclear "adequate" volume, not to exceed 1% of the total blood volume.[14,22] Using the ESCMID/ASM guidelines, we observed an overall increase in EFN documentation that was similar to the one observed by Kaditis et al. (12/113 and 22/123, respectively), the only published study that compared weight-adapted blood volumes to standard practice in pediatric hematology-oncology.[18] Importantly, six episodes could have been missed without the culture of large blood volumes (4−6 bottles), as highlighted by the low number (1 or 2) of positive bottles (EFN 19-21-25-36-37-38, Table 3). Accordingly, the volume to collect per weight group should be made more explicit in guidelines for managing pediatric hematology-oncology patients.

Benefits of the single-sampling strategy

The strategy was first described in adult patients[12,23] to decrease contamination rate, to control the risk of omitting the sampling of BC sets that impacts on BC yield, to improve patient comfort and to begin earlier antimicrobial treatment. It was also justified by the fact that bacteremia is exceptionally intermittent.[24] In children with hematologic malignancies, it is also particularly justified by the critical need to administer antibiotics at the earliest point, and to minimize handling of intravascular devices. Because Kaditis et al. performed two samples, each of approximately 1% of total blood volume, within a close time (10 min),[18] we assumed that these volumes could be safely drawn at once. Blood volume removal represented <1.5% (except for the 11 patients who weighted 14–18.9 kg for whom blood loss ranged from 1.6 to 2.5%) in our study. No adverse effects were observed, which suggest that limits advanced in guidelines can be re-assessed.

Importantly, EFN were documented earlier during period 2, with a double number of BSI diagnosed at day 1 of fever despite the rate of shortened diagnosis did not reach significance due to the small sample size. In addition, there were more positive bottles per documented EFN, which strengthened diagnosis confidence, particularly when a low-virulent opportunistic pathogen was recovered. Contaminant recognition with the single-sampling strategy can rely on the number of positive bottles, although this requires further reinforcement in pediatric and/or in hematology-oncology populations.[25] Whereas more bottles were positive, there was an overall 22% reduction in the number of BC sets sampled during period 2. This unexpected result may be explained at least in part by the fact that patients with documented EFN were subsequently less drawn for BC and underlines that the strategy not only improved BC yield but also contributed to improve overall blood sparing and to minimize handling of intravascular devices.

Blood culture yield over time

Despite the guidelines that recommend collecting BCs for the first 3 days of EFN in the clinically stable oncology patients with persistent fever, many physicians send daily BC sets beyond 3 days.[14] In our study, no episode was documented beyond day 4 of fever, whatever the strategy adopted, which is consistent with other studies.[26–28]

In Rosenblum et al.'s study, bacteremia was detected in 10.9% of EFN from patients with an initial negative BC when a repeat BC was obtained. Risk factors were with a previous history of bacteremia or hospitalization for more than 48 h prior to the onset of fever.[26] In Petty et al.'s study, all patients who had late positive BC experienced clinical changes or a new infectious episode.[28] Wattier et al. showed that BCs collected on day 1 of fever documented 11.5% of EFN vs. 1.5% per day for BCs collected after day 1 while the incidence of BC contamination was 1.1% per day. The majority of BC utilization occurred during the time of lowest diagnostic yield, and patients were subject to additional risks.[27] The authors argued for reducing the number of BCs performed after 24 h of fever. When the volume of blood is optimized on the first sample, the false-negative rate of initial BCs may be lower, thereby leading to lower rates of apparently new BSI identified subsequently.[11,22,27]

These promising results show undeniable benefits of combining single-sampling of a large volume of blood and weight-adapted blood culture strategies during hematologic malignancies. Bacteremia detection and time to microbial documentation were both improved,

and were associated with an overall blood sparing and reduced handling of intravascular devices. Feasibility now established, this blood sampling strategy warrants an expanded evaluation of the actual diagnostic yield increase in children through a multicentre clinical trial. More generally, performing a similar evaluation is crucial in the overall pediatric population suspected for BSI.

ACKNOWLEDGEMENTS

This work was supported by the University Hospital of Montpellier.

AUTHOR CONTRIBUTIONS

B.L., H.M. and N.S. conceptualized and designed the study, carried out the analyses, and drafted reviewed and revised the manuscript. S.D. collected the data, carried out the analyses, drafted the initial manuscript, and critically reviewed the manuscript. L.S. and S.H. conceptualized and designed the study, collected the data, and reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

Informed consent: This study is an observational study that fell within routine practice with nonadditional diagnostic and monitoring procedures applied to the patient. During period 1 data derived from routine clinical care were retrospectively analyzed. During period 2, the unit switched its blood culture sampling strategy; data were prospectively collected but strictly corresponded to the routine care of the patients with no additional intervention. Thus, research concerns only data derived from the routine care of the patient. In this context, the Ethics Advisory Committee waived the requirement to obtain a signed informed consent document, as accepted for research procedures involving no risk for the patient.

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REFERENCES

1. Ardura, M. I. & Koh, A. Y. in *Principles and Practice of Pediatric Oncology 7th Edition* (eds Pizzo, P. & Poplack, D.) 1010−1057 (Lippincott Williams & Wilkins, Riverwoods, 2015).
2. Hakim, H., Flynn, P. M., Knapp, K. M., Srivastava, D. K. & Gaur, A. H. Etiology and clinical course of febrile neutropenia in children with cancer. *J. Pediatr. Hematol. Oncol.* **3**, 623–629 (2009).
3. Castagnola, E. et al. A prospective study on the epidemiology of febrile episodes during chemotherapy-induced neutropenia in children with cancer or after hemopoietic stem cell transplantation. *Clin. Infect. Dis.* **45**, 1296–1304 (2007).
4. Meckler, G. & Lindemulder, S. Fever and neutropenia in pediatric patients with cancer. *Emerg. Med. Clin. North Am.* **27**, 525–544 (2009).
5. Isaacman, D. J., Karasic, R. B., Reynolds, E. A. & Kost, S. I. Effect of number of blood cultures and volume of blood on detection of bacteremia in children. *J. Pediatr.* **128**, 190–195 (1996).
6. Kellogg, J. A., Manzella, J. P. & Bankert, D. A. Frequency of low-level bacteremia in children from birth to fifteen years of age. *J. Clin. Microbiol.* **38**, 2181–2185 (2000).
7. Lamy, B., Dargère, S., Arendrup, M. C., Parienti, J. J. & Tattevin, P. How to optimize the use of blood cultures for the diagnosis of bloodstream infections? A state-of-the art. *Front. Microbiol.* **7**, 697 (2016).
8. Huber, S., Hetzer, B., Crazzolara, R. & Orth-Höller, D. The correct blood volume for paediatric blood cultures: a conundrum? *Clin. Microbiol. Infect.* **26**, 168–173 (2020).
9. Cuenca-Estrella et al. ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: diagnostic procedures. *Clin. Microbiol. Infect.* **18**(Suppl 7), 9–18 (2012).
10. Lamy, B. & Seifert, H. in *European Manual of Clinical Microbiology (SFM/ESCMID)* 1st edn (eds Courcol, R. et al.) 15−20 (SFM, Paris, 2012).
11. Baron, E. J. et al. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). *Clin. Infect. Dis.* **57**, e22–e121 (2013).
12. Lamy, B., Roy, P., Carret, G., Flandrois, J. P. & Delignette-Muller, M. L. What is the relevance of obtaining multiple blood samples for culture? A comprehensive model to optimize the strategy for diagnosing bacteremia. *Clin. Infect. Dis.* **35**, 842–850 (2002).
13. Lamy, B., Sundqvist, M. & Idelevich, E. A. Bloodstream infections—standard and progress in pathogen diagnostics. *Clin. Microbiol. Infect.* **26**, 142–150 (2020).
14. Lehrnbecher, T. et al. Guideline for the management of fever and neutropenia in children with cancer and/or undergoing hematopoietic stem-cell transplantation. *J. Clin. Oncol.* **30**, 4427–4438 (2012).
15. Hentrich, M. et al. Central venous catheter-related infections in hematology and oncology: 2012 updated guidelines on diagnosis, management and prevention by the Infectious Diseases Working Party of the German Society of Hematology and Medical Oncology. *Ann. Oncol.* **25**, 936–947 (2014).
16. Barton, C. D., Waugh, L. K., Nielsen, M. J. & Paulus, S. Febrile neutropenia in children treated for malignancy. *J. Infect.* **71**(Suppl 1), S27–S35 (2015).
17. Averbuch, D. et al. Antimicrobial resistance in Gram-negative rods causing bacteremia in hematopoietic stem cell transplant recipients: intercontinental prospective study of the Infectious Diseases Working Party of the European Bone Marrow Transplantation group. *Clin. Infect. Dis.* **65**, 1819–1828 (2017).
18. Kaditis, A. G., O'Marcaigh, A. S., Rhodes, K. H., Weaver, A. L. & Henry, N. K. Yield of positive blood cultures in pediatric oncology patients by a new method of blood culture collection. *Pediatr. Infect. Dis. J.* **15**, 615–620 (1996).
19. Dien Bard, J. & Tekippe, E. M. Diagnosis of bloodstream infection in children. *J. Clin. Microbiol.* **54**, 1418–1424 (2016).
20. Gaur, A. et al. Optimizing blood culture practices in pediatric immunocompromised patients: evaluation of media types and blood culture volume. *Pediatr. Infect. J.* **22**, 545–552 (2003).
21. Szymczak, E. G., Barr, J. T., Durbin, W. A. & Goldmann, D. A. Evaluation of blood culture procedures in a pediatric hospital. *J. Clin. Microbiol.* **9**, 88–92 (1979).
22. Freifeld, A. G. et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. *Clin. Infect. Dis.* **52**, e56–e93 (2011).
23. Arendrup, M., Jensen, I. P. & Justesen, T. Diagnosing bacteremia at a Danish hospital using one early large blood volume for culture. *Scand. J. Infect. Dis.* **28**, 609–614 (1996).
24. Li, J., Plorde, J. J. & Carlson, L. G. Effects of volume and periodicity on blood cultures. *J. Clin. Microbiol.* **32**, 2829–2831 (1994).
25. Leyssene, D. et al. Species-driven interpretation guidelines in case of a single-sampling strategy for blood culture. *Eur. J. Clin. Microbiol. Infect. Dis.* **30**, 1537–1541 (2011).
26. Rosenblum, J., Lin, J., Kim, M. & Levy, A. S. Repeating blood cultures in neutropenic children with persistent fevers when the initial blood culture is negative. *Pediatr. Blood Cancer* **60**, 923–927 (2013).
27. Wattier, R. L., Dvorak, C. C., Auerbach, A. D. & Weintrub, P. S. Repeat blood cultures in children with persistent fever and neutropenia: diagnostic and clinical implications. *Pediatr. Blood Cancer* **62**, 1421–1426 (2015).
28. Petty, L. A. et al. Repeated blood cultures in pediatric febrile neutropenia: would following the guidelines alter the outcome? *Pediatr. Blood Cancer* **63**, 1244–1249 (2016).