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Original Article

False Positive Peripheral Blood Cultures in Children with Leukaemia: A Descriptive Retrospective Prevalence Study

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ABSTRACT

Aim: False positive blood cultures are one of the critical quality indicators in healthcare services and high rates cause severe quality problems. This study aimed to determine the rate of false positive peripheral blood cultures (FPPBC) and possible associated factors in children with leukaemia.

Materials and Methods: A descriptive observational study was conducted with data from children hospitalised in the haematology oncology clinic of a children's hospital between March 2021 and March 2024. The results of those children who underwent peripheral blood cultures in routine care were collected using the "case report form" and "peripheral blood culture evaluation form-children" by analysing the electronic medical records.

Results: In this study, 1,003 peripheral blood culture results from 100 patients were followed up. Of these, 90 (8.9%) were defined as positive blood cultures. Of these, 27 (2.69%) were FPPBC The most common contaminants were *Staphylococcus epidermidis* (n=31) and *Staphylococcus hominis* (n=6). The highest seasonal contamination rates were observed in winter (29.6%), and during the daytime shift (48.1%).

Conclusion: The false positive peripheral blood culture rate was found to be within the optimal range in this study. It may be effective in reducing the contamination rates when infection controllers and educator nurses make the right interventions and provide training prepared in line with the guidelines. It should be highlighted that false positivity in peripheral blood culture collection is an important health and quality problem, and therefore, awareness-raising and training activities should be continued among nurses performing these collections.

Keywords: Children, false-positive, leukaemia, peripheral blood culture

Introduction

Leukaemias are the most common malignancy of childhood. The main treatment for childhood leukaemia is chemotherapy (1,2). Leukemic children frequently encounter bloodstream infection (BSI) due to the immunosuppressive side effects of chemotherapy treatment (3). BSIs are defined as the primary cause of morbidity and mortality in children with leukaemia. Therefore, early diagnosis of BSI ***via*** blood culture and the initiation of appropriate treatment are of vital importance (4). Blood culture obtained by peripheral venipuncture is essential in diagnosing the causative agent of the infection, especially in immunosuppressed patients.

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It is especially used for the diagnosis of invasive infections characterized by fever and/or other signs of sepsis (5). The growth of a microorganism in one blood culture set (aerobic and anaerobic) may mean that the patient has an infection. It is important to determine the microorganisms present as quickly as possible in order to reveal whether they are causative or contaminant, to perform antibiotic susceptibility tests of the microorganism accepted as causative and to direct the treatment correctly so as to reduce mortality and morbidity (6).

Despite today's advanced technologies, blood culture has remained the gold standard for the detection of BSIs for many years. Failure to follow the necessary techniques during culture collection may result in false-positive blood cultures in some cases. A positive peripheral blood culture a positive peripheral blood culture result may also be seen in the event of contamination with the skin flora of the person performing the blood culture collection (7). False positive peripheral blood cultures (FPPBC) are a single-culture bottle containing microorganisms belonging to the skin flora (8). False positive results can lead to many unfavourable situations (9). FPPBC may cause prolonged hospital stays, additional tests, unnecessary exposure of the patient to antimicrobial agents, the development of antibiotic resistance, increased risk of other infections, and increased hospital costs (10-12).

FPPBC infection rates may vary according to clinical settings. Most studies in the literature are conducted in emergency departments and are not associated with any underlying disease, and FPPBC rates vary between 1% and 10% in different studies (13). However, as recommended by the Clinical Laboratory Standards Institute (CLSI), the rate of blood culture contamination should not exceed 3% (6,14). However, some studies have reported that this rate may be around 10% (15,16). Since approximately 20% of microorganisms can persist despite skin antisepsis, it is not possible to eliminate this rate (17). Many internationally accredited clinical laboratories routinely calculate and report the institution's blood culture contamination rate as a quality assurance indicator. Rates are regularly reported to infection prevention, antibiotic stewardship and nurse/phlebotomy teams in order to target improvements in this pre-analytic quality indicator (18,19).

Purpose of this Study

In this context, this study aimed to determine the prevalence of FPPBC by retrospectively examining the peripheral blood culture results obtained during routine follow-ups from children hospitalized with leukaemia in a pediatric haematology/oncology (PHO) in-patient setting.

Materials and Methods

Study Design, Setting, and Sampling

This descriptive, observational, single-centred retrospective chart study was conducted using data from pediatric patients treated for leukaemia as inpatients in the PHO clinic of a tertiary university hospital between March 2021 and March 2024. The Strengthening the Reporting of Observational Studies in Epidemiology (20) guideline was adhered to throughout this research process. The PHO clinic consists of 17 beds.

Participants

No sample selection was made in this study, and all children who were newly diagnosed within three years were included (n=106). Those who had never had a peripheral blood culture during hospitalisation (n=5) and those who had a disease other than leukaemia (n=1) were excluded from this study. As a result, a total of 100 patients were included in the final analysis.

The inclusion criteria were that the children were between 0-18 years of age, did not have any disease other than leukaemia, received inpatient chemotherapy treatment at the PHO within the three-year period, had a peripheral blood culture test, and the culture results were accessible through the electronic medical records of the hospital. The exclusion criteria included those patients who had never had a peripheral blood culture taken and those diagnosed with a disease other than leukaemia.

Definitions

After analysing the electronic medical records of the hospital, the investigators in the research team decided together with the infection control team whether all peripheral blood culture results reported as positive were true positive (true BSI) or false positive (contamination). According to previous studies in this field (21,22), we defined the criteria for true BSI as follows:

• ***Staphylococcus aureus,*** gram-negative bacilli or Candida species were considered true positive if isolated from any bottle; skin contaminants [coagulase negative *Staphylococcus* (*Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus capitis, Staphylococcus haemolyticus*), *Viridans streptococci, Aerococcus, Micrococcus, Propionibacterium* spp., *Bacillus* spp., (*B. Anthracis*), *Corynebacterium* spp., and alpha-gamma haemolytic streptococci, *Dermabacter* spp, *Rothia* spp., and *Kocuria* spp.,] were isolated from at least two bottles collected from different sites and were considered true positive if the

patient had a high body temperature (>38.3 °C), chills or hypotension (systolic blood pressure <90 mmHg) and any of these microorganisms were also present in the patient's port catheter culture (all children in the sample also had a port catheter). The results of those children who had the same microorganism grow in both the peripheral culture and the catheter culture taken at the same time were considered true positives. Skin contaminants were defined according to the Centers for Disease Control and Prevention/National Healthcare Safety Network commensal list (21). When these criteria were insufficient, the assessors reviewed the clinical information to judge the results.

Peripheral Blood Culture Collection Practices at the Study Institution

Preparation: In Ege University Children's Hospital Pediatric Haematology Clinic, the necessary materials are prepared after a physician's order to perform this procedure. The procedure is explained to the child and parents. It was observed that the nurse who performed the culture collection generally washed her hands.

Skin antisepsis: In the institution, skin antisepsis is provided with the available antiseptic agent. This agent can be 70% isopropyl alcohol, 10% povidone-iodine or 2% chlorhexidine gluconate +70% isopropyl alcohol. In the establishment of skin antisepsis, the technique of rubbing back and forth or cleaning from the centre outwards was generally not used, and the area to be cultured was wiped from top to bottom several times. It was observed that not all nurses waited for the appropriate drying time after skin antisepsis. Non-sterile gloves were mostly used during culture collection.

Time and number of cultures taken: In the clinic, peripheral blood cultures are taken, especially when patients show signs of fever and tremor, when signs of sepsis are present and also before antimicrobial therapy is started. If the patient already has ongoing antibiotherapy, the rule that it should be taken immediately before the next dose may be ignored.

Blood volume: Especially in children receiving chemotherapy, intravenous interventions may be more difficult due to a deterioration of vascular structures, the presence of subcutaneous tissue around the vessels and/or problems in communicating with children. Therefore, in some cases, the required blood volume of 3-5 mL may not be reached.

Culture collection technique: In the clinic, blood cultures were taken by clinical nurses. The clinic did not have a special phlebotomy team. In cases where palpation could not be performed properly, it was observed that the person taking the culture touched the skin again after skin antisepsis. The rubber caps of the culture bottles were wiped with 70% isopropyl alcohol before occlusion of the blood samples. No special peripheral culture collection technique (e.g. the aseptic non-touch technique) was applied.

Data Collection Procedure

In this study, we assessed all peripheral blood culture results of those children who met the inclusion criteria. The relevant data was obtained by accessing the medical records of the hospital. The protocol numbers of the eligible children were determined, and their relevant records were assessed and recorded in the data collection forms.

Data Collection Forms

Case report form: This form consists of different questions determining the socio-demographic characteristics (sex, age, diagnosis, risk group, relapse status) of the children.

Peripheral blood culture evaluation form-children: This form was developed by the researchers in line with the literature (22,23). It included the dates of the peripheral blood culture of the child, the exact time of the culture, the growth status of the peripheral blood culture, the name of the microorganism which grew, and the contamination status.

Ethics

This study was conducted following the Declaration of Helsinki and approved by the Ege University Medical Research Ethics Board on (approval no.: 24-3T/48, date: 07.03.2024) and study permission was obtained from the hospital where this study was conducted. As the study had a retrospective design and the data were accessed through the hospital's electronic medical records, the requirement for written informed consent was waived. The study protocol was registered at ClinicalTrials.gov: NCT06336837. https://clinicaltrials.gov/study/NCT06336837.

Statistical Analysis

All the statistical analysis was performed using the IBM Statistical Package for Social Sciences program version 25.0. A descriptive analysis was conducted for demographic, clinical characteristics, and microbial organisms. Categorical variables are presented as frequencies and percentages. Mean ± standard deviation was computed for continuous data. Statistical analyses were performed using the proportions of FPPBC with respect to the shifts and

dates when they were carried out using cross tabulations. The chi-square test was used to compare these proportions. The FPPBC rate was calculated by dividing the total number of contaminated blood cultures by the total number of cultures and multiplying by 100 (6). In this study, logistic regression (LR) analysis was also performed in order to evaluate the factors affecting FPPBC.

Results

Characteristics of the Participants

The mean age of the children in this study was 7.65±4.74 years. Most of the children were male (62%) and most of them were diagnosed with acute lymphoblastic leukaemia (ALL) (85%). More than half of the children were receiving chemotherapy in accordance with the high-risk group protocol (55%) and 75% were receiving leukaemia treatment for the first time (Table I).

Characteristics of the Blood Cultures

Of the 1,003 peripheral blood cultures, 90 (8.9%) were defined as positive blood cultures showing microbial growth. Of these, 43 were blood culture contaminations, representing 2.69% of the total blood cultures and 30% of the positive blood culture samples. The most prevalent contaminant species were *Staphylococcus epidermidis* (66.7%) and *Staphylococcus hominis* (14.8%). The other species which were identified are shown in Table II.

Possible Related Factors

When the distribution of FPPBC was analysed in the results, it was found that FPPBC occurred most frequently during the daytime shift (48.1%). When the seasonal difference was investigated, it was found that FPPBC was most frequently seen in the winter months (29.6%). However, neither difference was statistically significant (p=0.236 and p=0.939, respectivel) (Table III).

The enter method was used to determine the final model factors affecting FPPBC. According to this model, neither risk group nor relapse status had a statistically significant effect on the development of FPPBC. On the other hand, the sex and the diagnosis of the participants were found to be effective on FPPBC. According to the model, the risk of FPPBC increased approximately 3-fold if the child had been diagnosed with ALL and was male (Table IV).

Table I. Descriptive characteristics of the participants

Descriptive characteristics of the children	**n**	**%**
Children's age mean (SD)	7.65 (4.74)	Min=1 Max=18
Children's gender		
Female	38	38
Male	62	62
Children's diagnosis		
Acute lymphoblastic leukaemia	85	85
Acute myeloblastic leukaemia	15	15
Risk group of children		
Standard risk group	26	26
Middle risk group	19	19
High risk group	55	55
Relapse status of children		
Yes	25	25
No	75	75

SD: Standard deviation

Table II. Microorganisms in positive and false-positive cultures

	n	**%**
Number of peripheral blood cultures	1,003	100
PPBC microorganisms		
Klebsiella pneumoniae	13	27.7
Escherichia coli	10	21.3
Enterococcus faecium	7	14.9
Stenotrophomonas maltophilia	6	12.8
Number of positive peripheral blood cultures	90	8.97
Number of false-positive peripheral blood cultures	27	2.69[a]
FPPBC microorganisms		
Staphylococcus epidermidis	18	66.7
Staphylococcus hominis	4	14.8
Streptococcus mitis/oralis	3	11.1
Streptococcus sanguinis	1	3.7
Staphylococcus haemolyticus	1	3.7

[a]: FPPBC rate was calculated by dividing the total number of contaminated blood cultures by the total number of cultures and multiplying by 100
FPPBC: False-positive peripheral blood cultures, PPBC: Positive peripheral blood cultures

Table III. Distribution of false-positive peripheral blood culture rates

	n	**%**	**Chi-square**	**p**
Shift of FPPBC collection				
08.00-15.59 (Day-shift)	13	48.1		
16.00-23.59 (Evening-shift)	8	26.9	2.889	0.236
00.00-07.59 (Night-shift)	6	22.2		
Date of FPPBC collection				
Winter	8	29.6		
Spring	6	22.2	0.407	0.939
Summer	6	22.2		
Autumn	7	25.9		

Chi-square test was used p<0.05.
FPPBC: False-positive peripheral blood cultures

Table IV. Evaluation of factors affecting false-positive peripheral blood cultures

Regression coefficients								
	B	**SE**	**Wald**	**Df**	**p**	**Exp(β)**	**90% CI for Exp(β)**	
							Lower	**Upper**
Constant	1.682	0.970	3.005	1	0.083	5.375	-	-
Sex								
Male[a]								
Female	1.171	0.653	3.215	1	0.073[b]	0.310	0.106	0.908
Diagnosis								
AML[a]								
ALL	1.118	0.571	3.837	1	0.050[b]	3.057	1.196	7.815
Risk group								
SRG[a]			2.689	2	0.261			
HRG	0.338	0.920	0.135	1	0.713	0.713	0.157	3.241
MRG	1.231	0.991	1.544	1	0.214	0.292	0.057	1.490
Relapse status								
Yes[a]								
No	0.403	0.773	0.272	1	0.602	0.668	0.187	2.383

Logistic Regression was used
[a]Reference, [b]p<0.10
FPPBC: False-positive peripheral blood cultures, ALL: Acute lymphoblastic leukaemia, AML: Acute myeloblastic leukaemia, HRG: High-risk group, MRG: Middle-risk group, SRG: Standard-risk group, SE: Standard error, Df: Degrees of freedom, CI: Confidence interval

Discussion

In this study, peripheral blood culture results obtained from 100 different children with leukaemia over a three-year period were retrospectively analysed. In our study, 62% of the participants were male and 85% were diagnosed with ALL. Out of the 1,003 peripheral blood cultures analysed in this study, 90 showed growth, and 27 of these were FPPBC (2.69%). ***Staphylococcus epidermidis*** (72.1%) and ***Staphylococcus hominis*** (14%) were the most commonly cultured microorganisms. This study found that FPPBCs were most commonly observed in cultures taken during the day shift during the winter months.

Although medical technologies are improving day by day, blood culture remains the most reliable method for the diagnosis for BSI. Even in such a reliable test, errors made during culture collection may result in inaccurate results (24). False results may have negative consequences on both the patient and the health care service. Therefore, the management of FPPBC, which is accepted as one of the quality indicators in health care in many developed countries, is a clinically important issue. This study aimed to retrospectively examine the results of peripheral blood cultures obtained from children hospitalised with leukaemia in the PHO clinic of a tertiary university hospital.

The fact that the majority of the participants of our study were male (62%) and their mean age was 7.65 (4.74) years was consistent with the literature. According to the American National Cancer Institute SEER data, the incidence of acute leukaemia in boys is 5.4/100.000, while it is 4.3/100.000 in girls and the median age is 6 years (2).

In this study, 1,003 peripheral blood culture results obtained between March 2021 and March 2024 were analysed through the electronic medical records of the hospital. Of the 1,003 peripheral blood cultures, 90 were found to have growth. True positive and false positive culture results were determined in accordance with the criteria specified in the definitions section of this study. FPPBC growth was detected in 27 of all cultures and this rate was calculated as being 2.69%, which is below the threshold rate as proposed by CLSI (<3%) (14). In a study conducted by Gorfinkel et al. (4) and colleagues with pediatric cancer patients, the FPPBC rate was reported to be 3.7%. Similarly, in the prevalence study conducted by Mullan et al. (25) in a pediatric emergency department, the rate of FPPBC was reported as being 3.17%. Likewise, the rate of FPPBC was reported to be 4.17% in the study by Aiesh et al. (26) in which the results of peripheral blood cultures obtained from all patients in a tertiary healthcare institution were analysed.

Staphylococcus epidermidis (72.1%) and ***Staphylococcus hominis*** (14%) were the most commonly cultured microorganisms. Similarly, ***Staphylococcus epidermidis*** (49.2%) was the most commonly grown microorganism in the study by Aiesh. The reason for the isolation of

Staphylococcus epidermidis in many studies is thought to be that this microorganism is the most common staphylococcal species on the skin (27). In recent years, the widespread use of medical devices and the excessive, incorrect or prolonged use of antibiotics and the ability of ***Staphylococcus epidermidis*** to adhere to smooth surfaces of different structures have caused this microorganism to emerge as an important nosocomial pathogen (28).

Also, in this current trial, FPPBC was most frequently seen in the peripheral blood cultures taken during the winter months (29.6%) and during the day shift (48.1%). Similarly, de Ponfilly et al. (29) indicated that the season in which peripheral blood cultures were most frequently obtained was winter. Although not statistically significant, it is thought that this situation is related to the fact that the clinical workload is more intense, especially in the winter months, and also that nurses work more heavily on the day shift. It is also thought that factors such as environmental conditions, mental workload, distraction and excessive stress, which are more intense during the day shift, may have led to an increase in implementation errors.

The method of obtaining the blood culture, the quality of the antiseptic solution, the gloves used in the culture collection, needle changes before inoculation, the training and experience of the health professional who perform the collection, the clinic's patient density, having nurses working under appropriate conditions, and regular notifications of peripheral blood culture contamination rates may support the clinic in keeping contamination levels low. For instance, in a study by He (30), the contamination rate was 4.96% (144/2903) in blood cultures collected by newly graduated nurses and 3.52% in blood cultures collected by senior nurses with more than five years of work experience. In our study, since the data were collected ***via*** an electronic medical record, it did not include data on the characteristics of the nurse who performed the blood culture collection. However, the fact that the majority of clinical nurses had more than five years of experience and that these clinical nurses were permanent were thought to be factors which reduced the rate of FPPBC.

In this study, factors which may affect the development of FPPBC were also evaluated by LR analysis. In the demographic data of our study, FPPBC was most common in the high risk group, with a rate of 55%. However, in LR analysis, it was found that the group most affected by the development of FPPBC was the standard risk group. This is contrary to the logic that the child with the highest neutropenia is exposed to more contamination. However, since FPPBC is one of the most important quality indicators and is basically a nursing care fault, a statistically significant difference between the false positive culture result and the diagnosis, risk group, and relapse status of the participants is not expected. LR analysis confirmed this expectation and showed that FPPBC was independent of risk group and relapse status.

In this study, the process steps of the nurses performing peripheral blood culture collection were also observed. Some problems were detected in these observations. The first of these was that not all nurses performed hand hygiene before culture collection. Other factors were the lack of proper technique in the application of the skin antiseptic and not waiting for the antiseptic to dry due to workload. Due to the difficulty of peripheral intervention in pediatric patients, it was also observed that the culture was taken with an insufficient amount of blood in some cases. Palpation after antisepsis and the lack of appropriate peripheral culture collection techniques were other root causes.

Study Limitations

This study addressed a critical issue by focusing on FPPBC and contamination obtained from a vulnerable population of leukemic children receiving chemotherapy in a PHO during routine care. The retrospective nature of this study allowed researchers to examine this subject as it occured in routine clinical care. Such patterned studies usually provide large study populations and longer observation periods, allowing for the examination of specific populations. In this study, researchers collected data over 3 years and examined over a thousand culture results. The findings provide information on a subject that is relatively less covered in national and international literature, especially with regards to revealing the frequency of false-positive peripheral blood cultures in pediatric patients with neutropenia. On the whole, this study can raise awareness of the false-positive blood culture rate, which is one of the most important health care indicators, and by extension, nursing care techniques.

While our study has the potential to be the first study to define and measure the rate of FPPBC in pediatric leukaemia patients in our country, it had some limitations, such as being a descriptive and single-centre study. The retrospective design of this study, the lack of data such as demographic data (education level, work experience) of the nurses who performed the culture collections which resulted in false positives, and the appropriateness of the techniques/materials used in the culture collection, etc. are the other limitations of this study. Further comparative studies with larger samples including multiple centres are recommended.

In addition to the results provided by this study, further retrospective and prospective studies are needed in order to study the rates of FPPBC and related factors in pediatric patients, especially in neutropenic and vulnerable populations. Performing larger, multi-centre studies will greatly improve the generalisability of these results, enable a more varied patient population to be included, and capture differences in care practices between different children's hospitals. In addition, the training of nurses who perform this collection process, regardless of whether it is peripheral or catheter culture, and the effect of other related factors on reducing contamination rates should also be investigated. Understanding how the knowledge, skills, and experience of nurses or other factors in the environment they work in (work shift, patient density, workload, etc.) affect contamination rates can lead to improved patient outcomes and higher healthcare quality. Finally, it is extremely important to use and validate a risk assessment tool specifically designed for pediatric populations. In particular, with the integration of policies and government organizations, the quality of healthcare service can be increased by taking into account the research recommendations listed above.

Conclusion

In this study, the most frequently observed microorganisms in the PHO clinic were found to be *Staphylococcus epidermidis* and *Staphylococcus hominis*. In addition, FPPBC was found to be higher during the winter months and on day shifts. In the analysis of the factors affecting the development of FPPBC, it was found that factors other than diagnosis and gender did not create a statistically significant difference.

The FPPBC rate was found to be lower than the maximum level recommended by CLSI. Although the FPPBC rate was within acceptable limits, appropriate and routine peripheral blood culture collection methods should be integrated into all patient care environments in order to prevent contamination and further reduce this rate.

In addition, within the scope of the education role, which is one of the most important professional roles of nurses, the awareness, knowledge and skill levels of nurses should be enhanced by using educational materials prepared in line with current guidelines and appropriate techniques. The main purpose of these trainings should be to improve the quality of nursing care so as to reduce or prevent contamination.

Ethics

Ethics Committee Approval: The study was conducted following the Declaration of Helsinki and approved by the ethics committee of Ege University Medical Research Ethics Board on (approval no.: 24-3T/48, date: 07.03.2024) and study permission was obtained from the hospital where the study was conducted.

Informed Consent: As the study had a retrospective design and data were accessed through the hospital's electronic medical records, the requirement for written informed consent was waived.

Footnotes

Authorship Contributions

Concept: S.A.S, F.Y., Design: S.A.S, F.Y., D.Y.K., Data Collection or Processing: S.A.S., N.K., Analysis or Interpretation: S.A.S., N.K., Literature Search: S.A.S., Writing: S.A.S, F.Y., D.Y.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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